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14. ABSTRACT
Ion mobility spectroscopy (IMS) allows one to differentiate between different isomers of a given molecular ion according to their collisional cross-section. Using two stages of IMS (IMS-IMS) one can select a specific isomer, collisionally heat it and follow its isomerization pathways. Recently it has been shown that this technique allows one to determine internal energy barrier for isomerization. Here we apply the technique to the important case of the retinal protonated Schiff base (RPSB). Photoisomerization of the RPSB is the primary in animal vision. We find that the energy barrier for a single *cis-trans* isomerization is 0.64 ± 0.05 eV, which is significantly lower than that observed for the reaction within opsin proteins. Thus the protein has a significant role in increasing the barrier energy for thermal isomerization relative to the gas phase which lacks interaction with the RPSB counterion and steric constraints. High barrier energy is mandatory for efficient vision processes, otherwise thermal noise would overwhelm the signal originating from the photochemical isomerization.

15. SUBJECT TERMS
ion mobility spectrometry, mass spectrometry, retinal, chromophore, isomerization energy

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Direct Measurement of the Isomerization Barrier of the Isolated Retinal Chromophore

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Synopsis Energy barrier Heights for isomerization of the isolated retinal chromophore were measured using two stages of ion mobility spectroscopy (IMS-IMS).

Ion mobility spectroscopy (IMS) allows one to differentiate between different isomers of a given molecular ion according to their collisional cross-section. Using two stages of IMS (IMS-IMS) one can select a specific isomer, collisionally heat it and follow its isomerization pathways (See Fig. 1). Recently it has been shown that this technique allows one to determine internal energy barrier for isomerization [1].

Here we apply the technique to the important case of the retinal protonated Schiff base (RPSB) [2]. Photoisomerization of the RPSB is the primary in animal vision. We find that the energy barrier for a single *cis*-*trans* isomerization is 0.64±0.05 eV, which is significantly lower than that observed for the reaction within opsin proteins. Thus the protein has a significant role in increasing the barrier energy for thermal isomerization relative to the gas phase which lacks interaction with the RPSB counter-ion and steric constraints. High barrier energy is mandatory for efficient vision processes, otherwise thermal noise would overwhelm the signal originating from the photochemical isomerization.

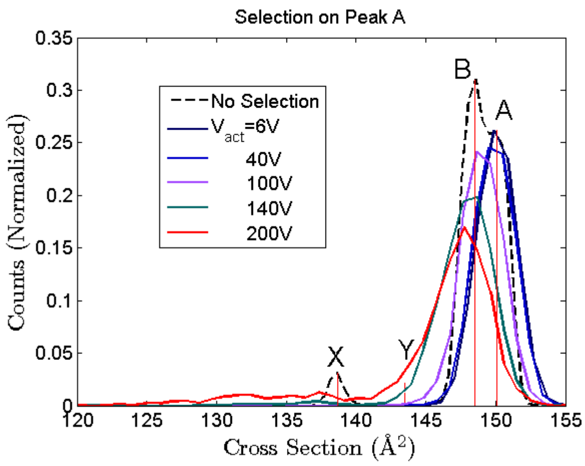


Figure 1. Results of selection and activation when the selection is applied to peak A (the *all-trans* isomer), and activation is performed for different activation voltages, V_{act}. The dashed line corresponds to the IMS of the RPSB with no selection.

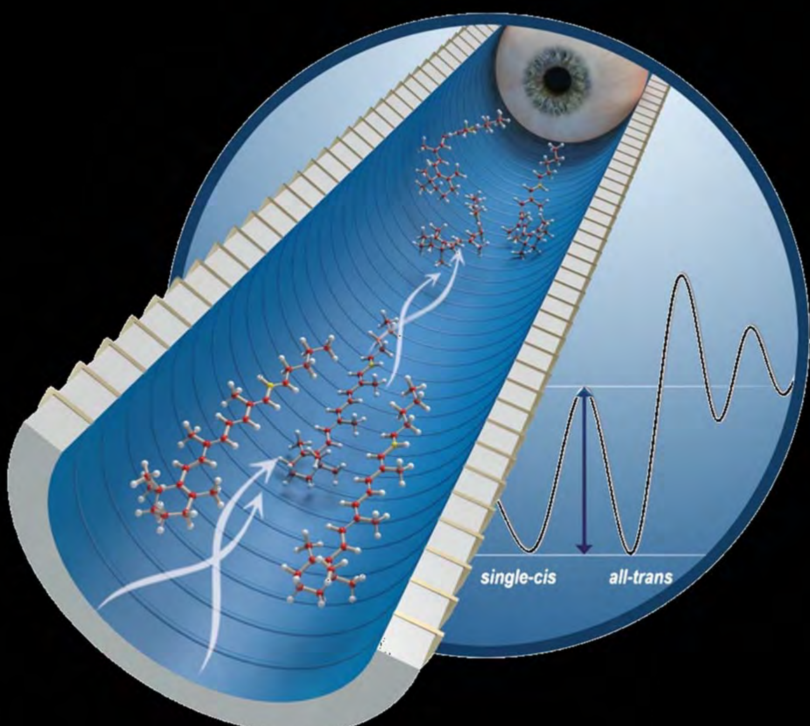
References

[1] N. A. Pierson, S. J. Valentine, D. Clemmer 2015 Int. J. Mass. Spectrom, **377**, 646-654.
[2] J. Dilger. Y. Toker *et al*. 2015 Ang. Chemie Int. Ed. **127**, 4830-4834.

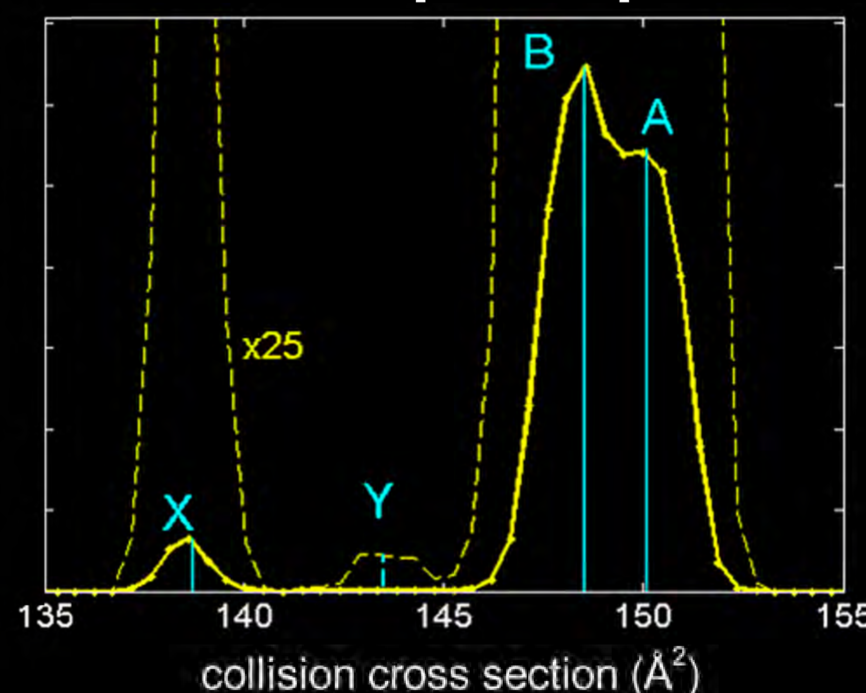
[1]E-mail: yonitoker@gmail.com

Direct Measurement of the Isomerization Barrier of the Isolated Retinal Chromophore

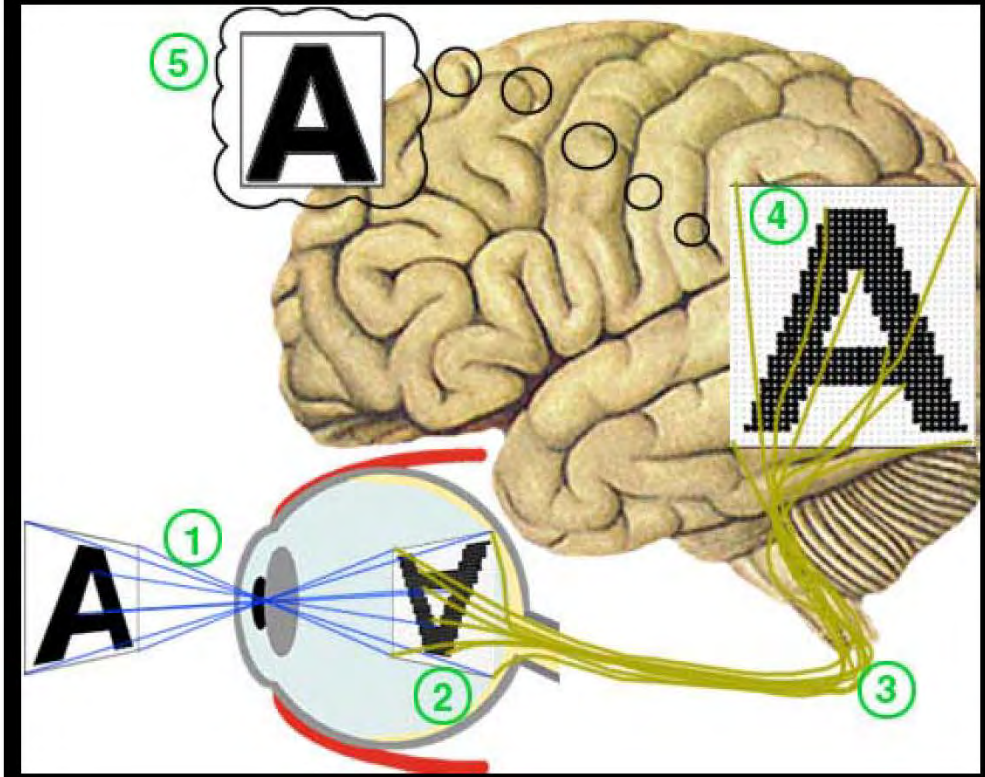
Dr. Jonathan M. Dilger
XXIX ICPEAC
July 23, 2015

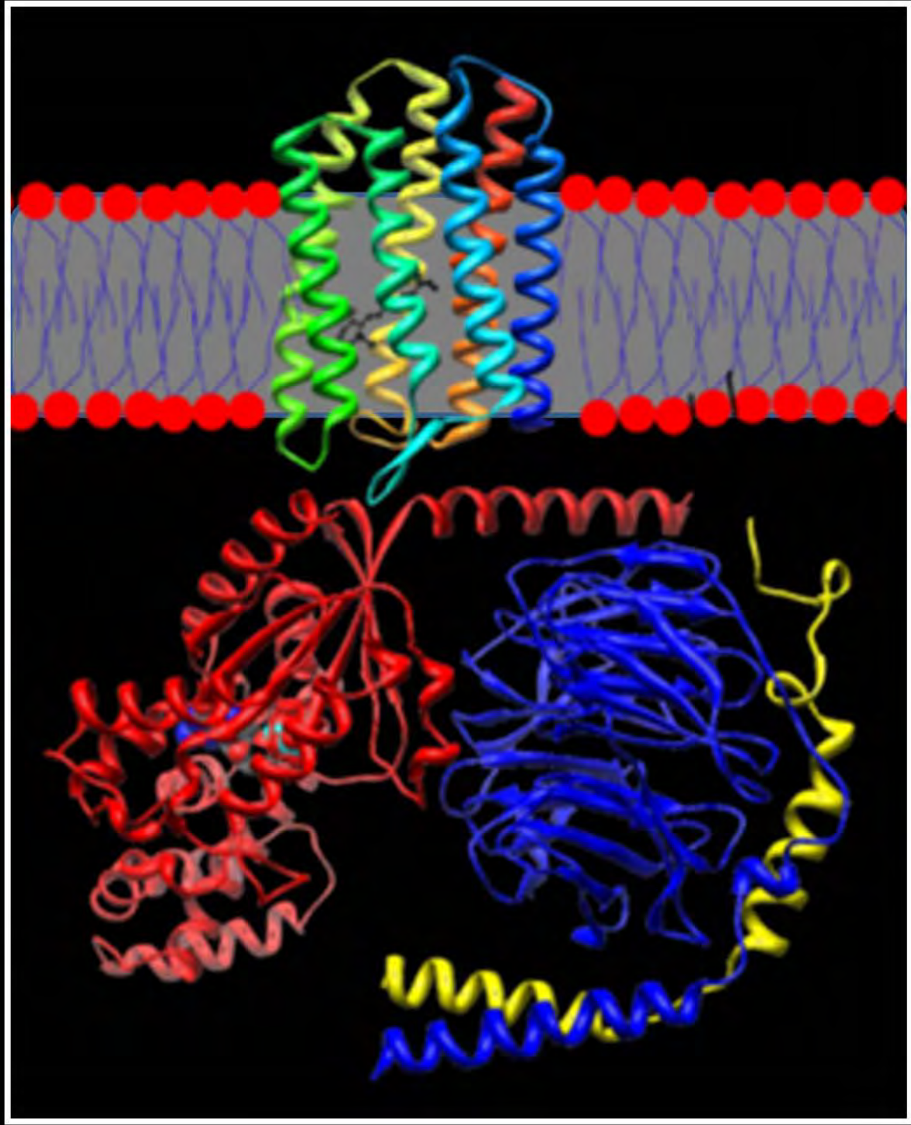


Retinal: $[RPSB+H]^+$



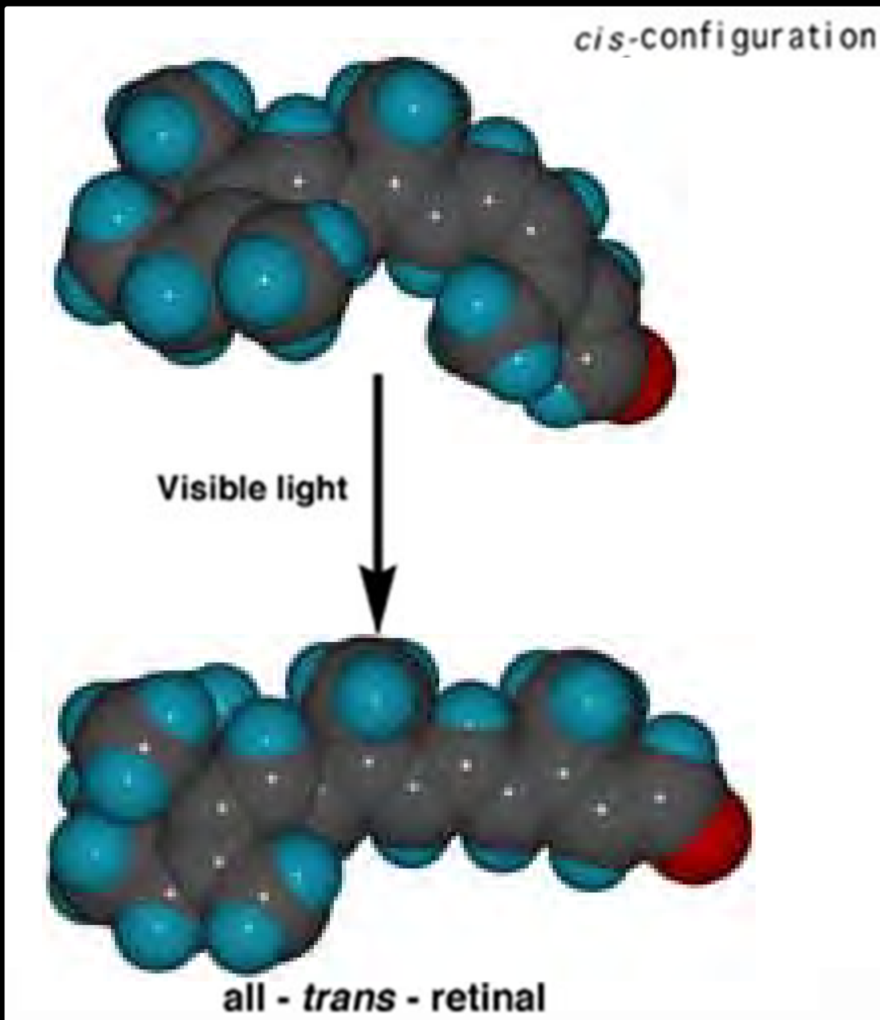
Vision

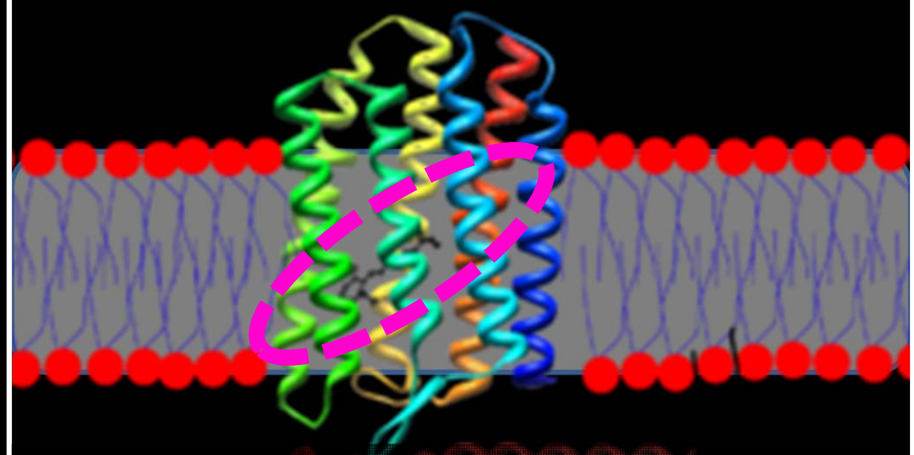


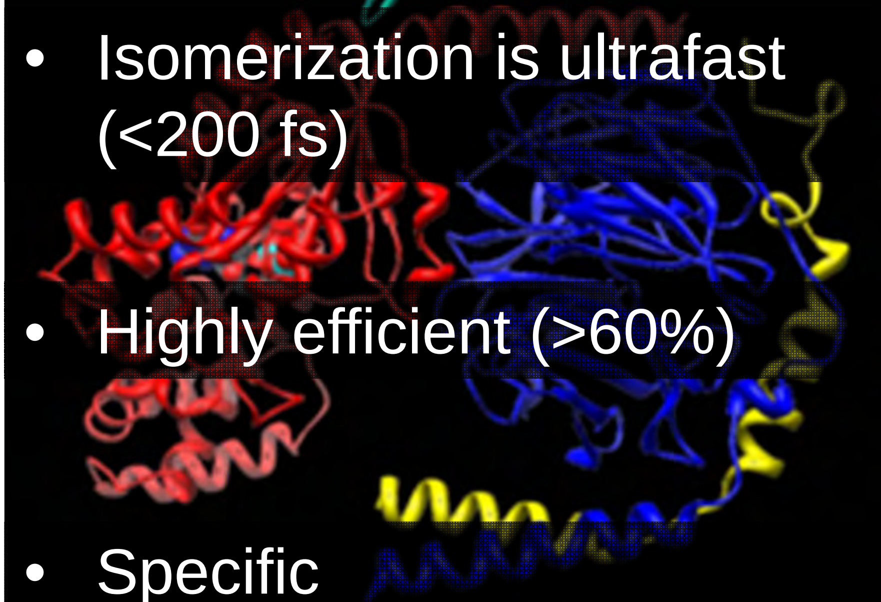


“Vision and Light-Induced Molecular Changes”,
www.chemistry.wustl.edu/~edudev/LabTutorials/Vision/Vision.html

Light-induced Retinal Isomerization





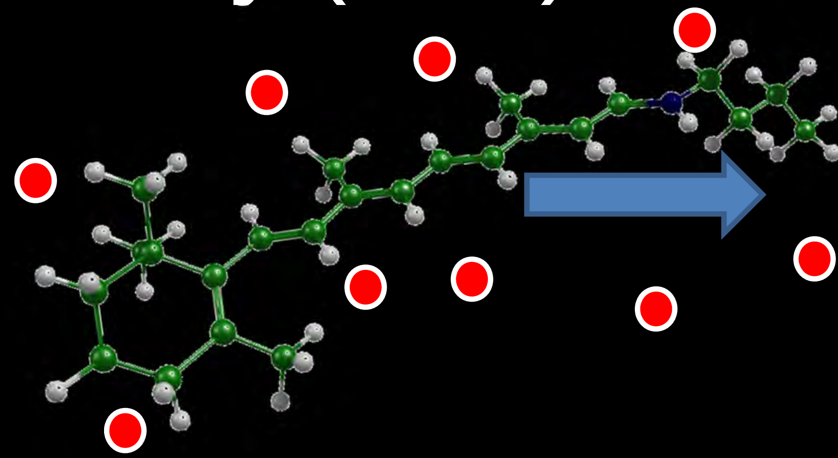


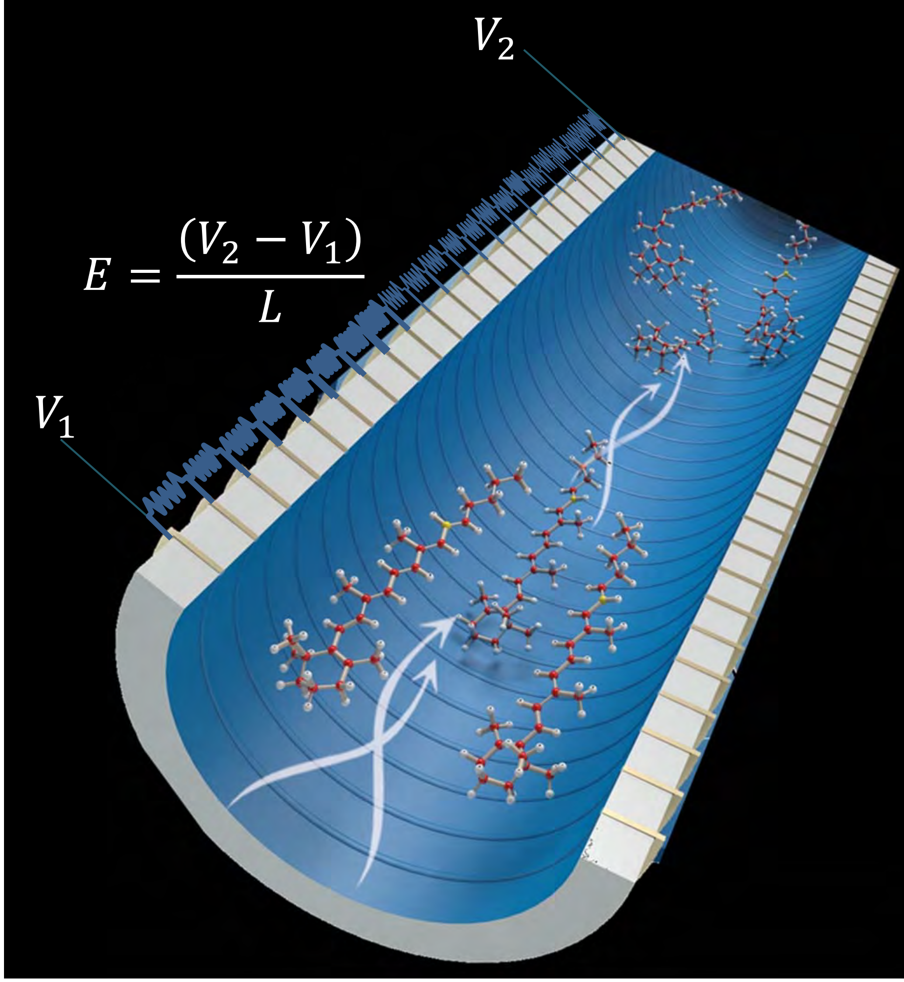
- Isomerization is ultrafast (<200 fs)
- Highly efficient (>60%)
- Specific

“Vision and Light-Induced Molecular Changes”, www.chemistry.wustl.edu/~edudev/LabTutorials/Vision/Vision.html

Ion Mobility Spectrometry (IMS)

IMS pulls molecular ions with a small electric field (typically ~10 V/cm) through a drift tube filled with gas





$$E = \frac{(V_2 - V_1)}{L}$$

The ions are accelerated by the electric field, and slowed down by the collisions, reaching an average drift velocity

$$v_d = \frac{L}{t_d} \qquad K = \frac{v_d}{E}$$

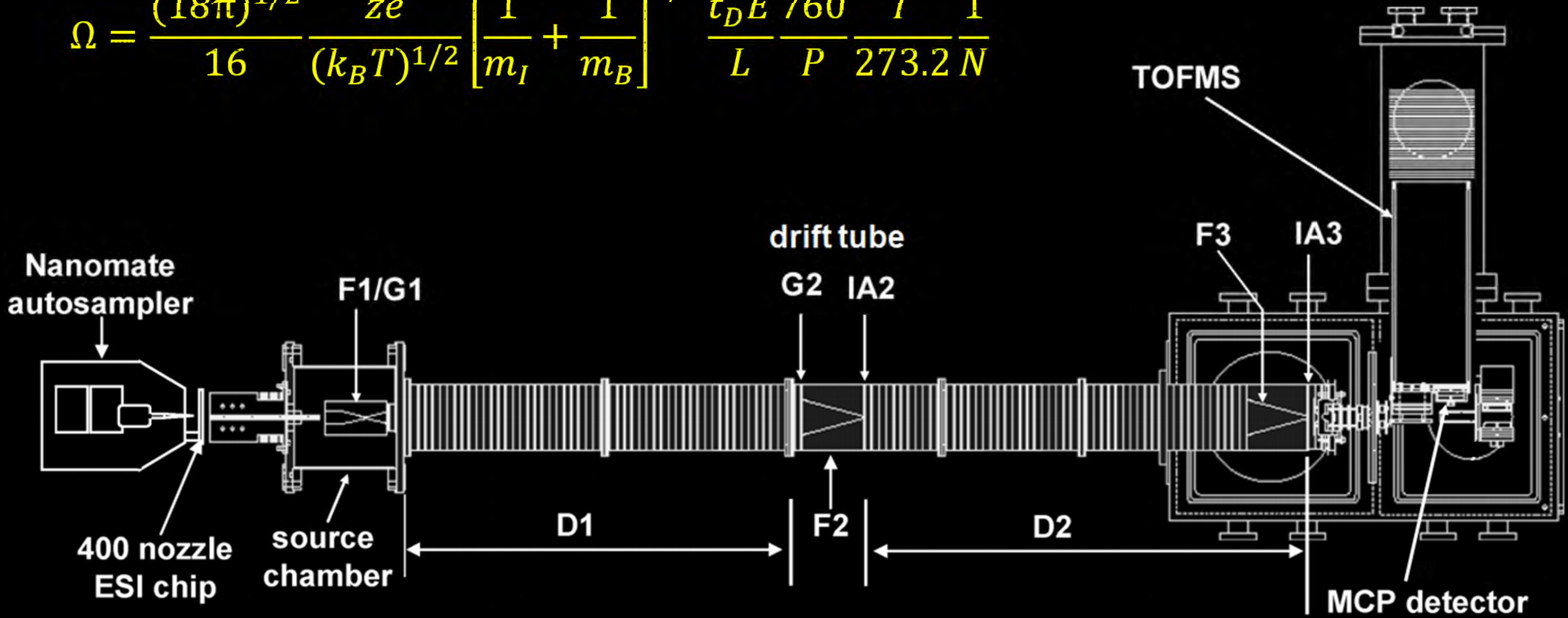
$$\mathrm{K} = \frac{1}{\Omega}\frac{\sqrt{18\pi}}{16}\frac{ze}{\sqrt{k_B T\,\mu}}\frac{1}{\mathrm{N(T,P)}}$$

Theory and Instrumentation

$$v_D = KE$$ Ion Mobility

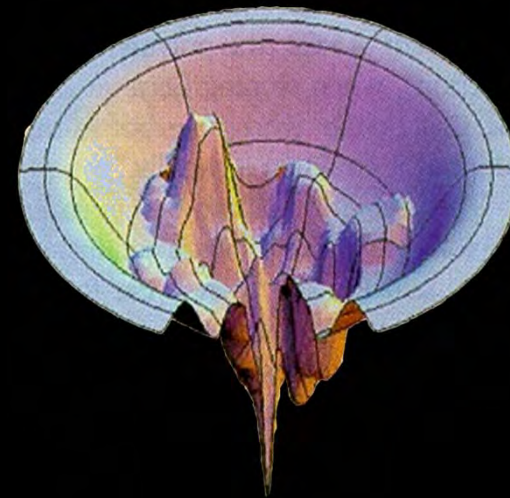
Collision cross section

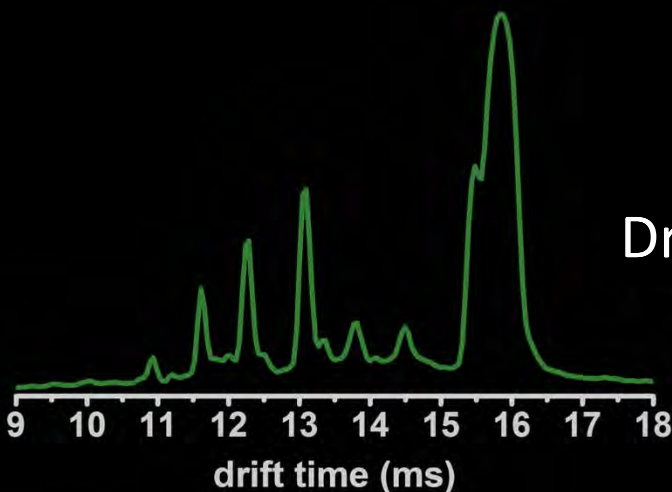
$$\Omega = \frac{(18\pi)^{1/2}}{16} \frac{ze}{(k_B T)^{1/2}} \left[\frac{1}{m_I} + \frac{1}{m_B}\right]^{1/2} \frac{t_D E}{L} \frac{760}{P} \frac{T}{273.2} \frac{1}{N}$$



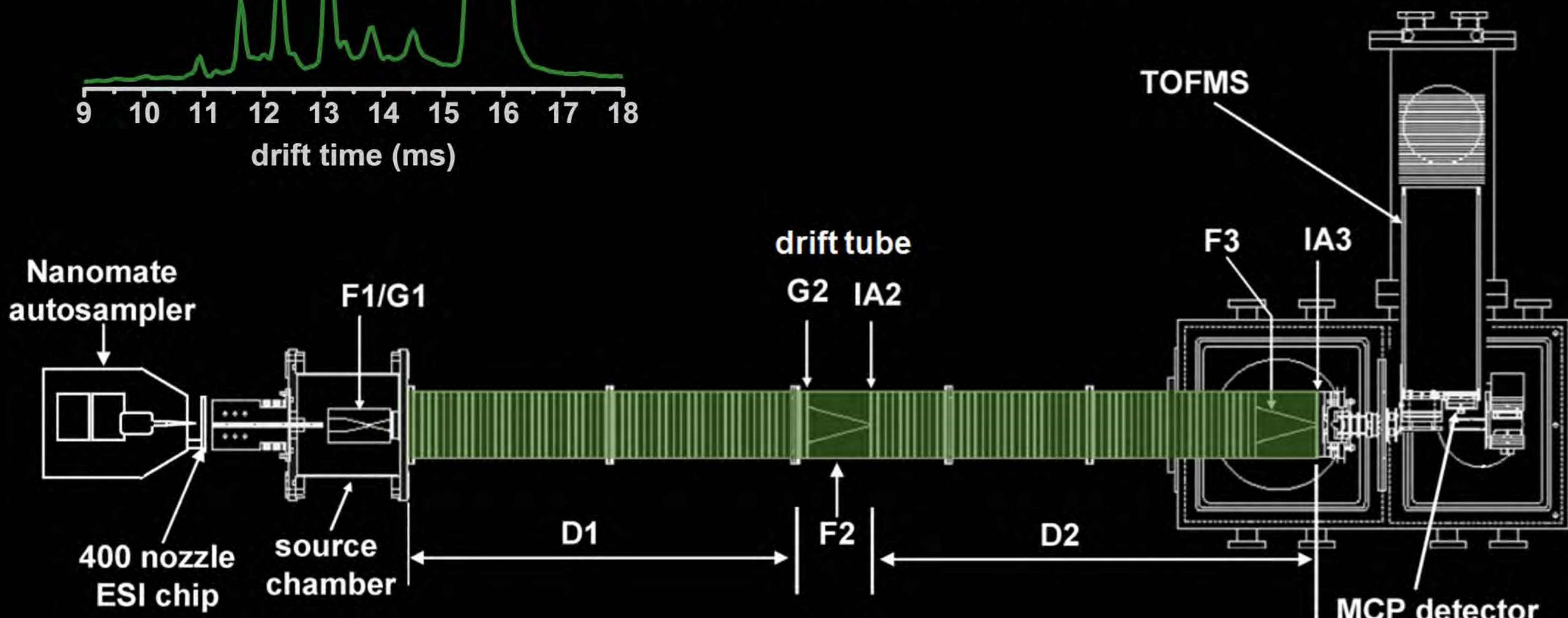
Revercomb, H. E.; Mason, E. A. *Anal. Chem.* **1975**, *47*, 970–983
Koeniger, et al. *Anal. Chem.* **2006**, *78*, 4161-4174

IMS-MS: Bradykinin (BK)



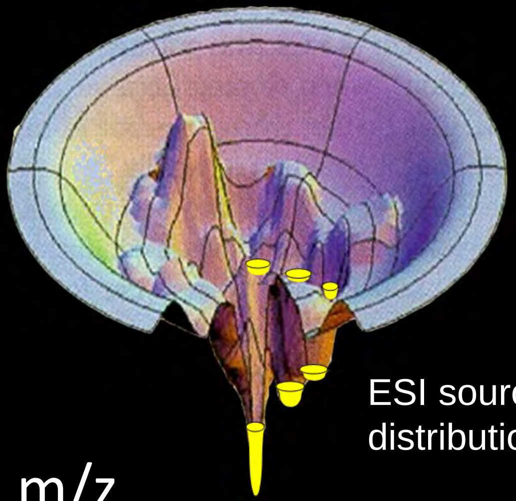


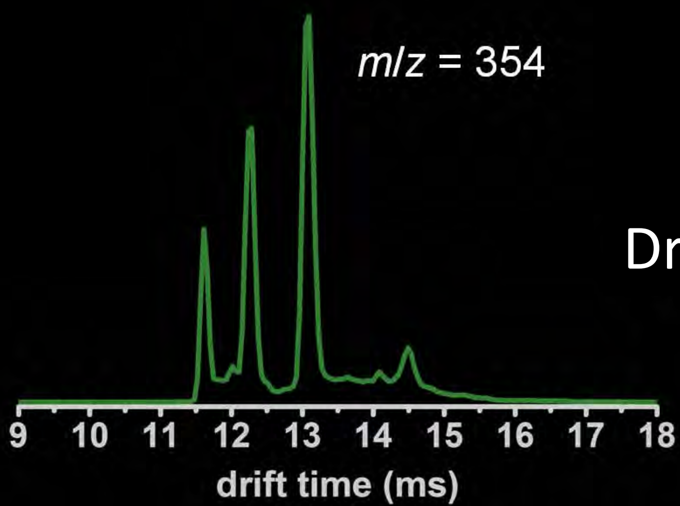
Drift profile of all ions



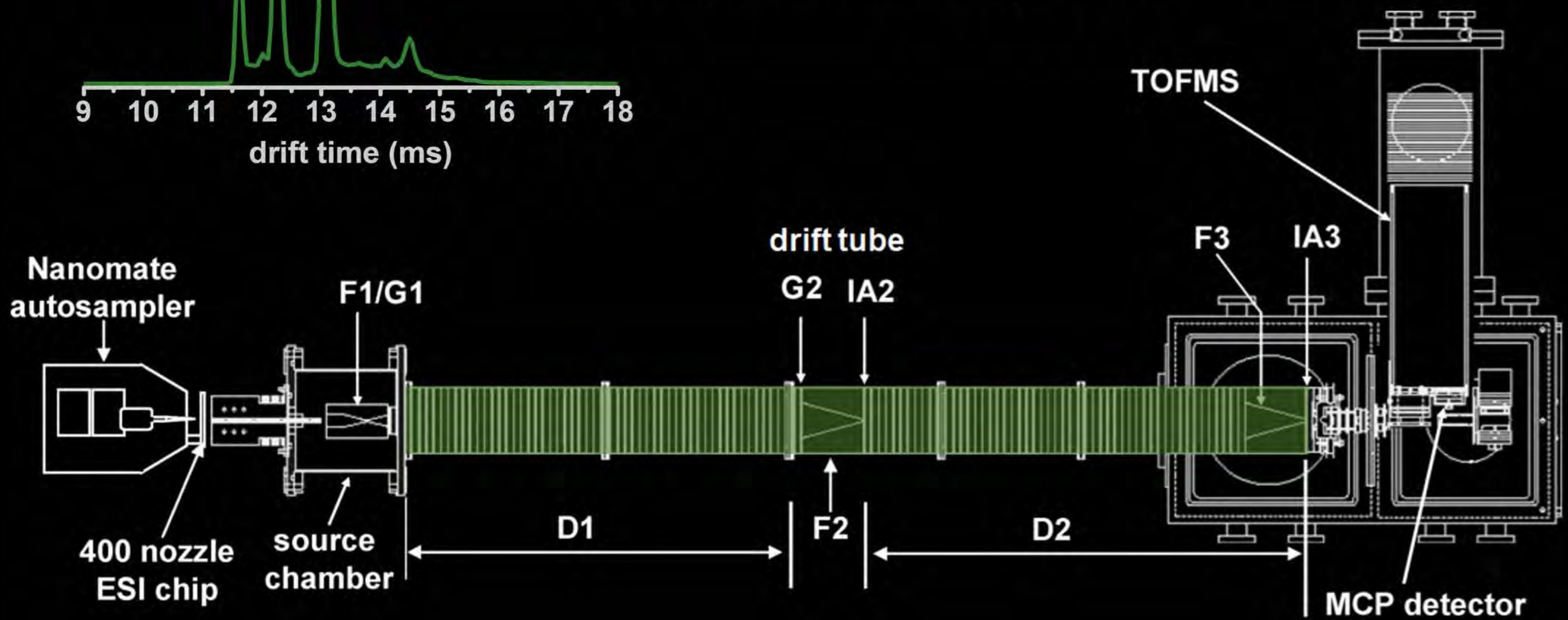
Surface modified from: Dill, K.A.; Chan, H. S. *Nature Structural Biology* **1997**, *4*, 10.

IMS-MS: $[BK+3H]^{3+}$





Drift profile of a single m/z



Surface modified from: Dill, K.A.; Chan, H. S. *Nature Structural Biology* **1997**, *4*, 10.

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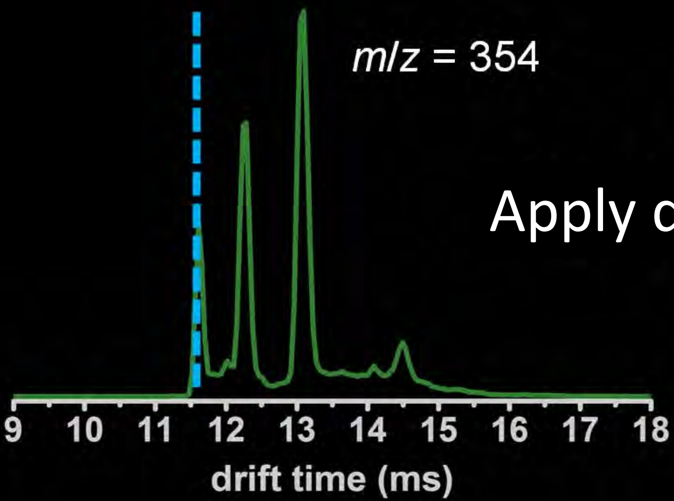
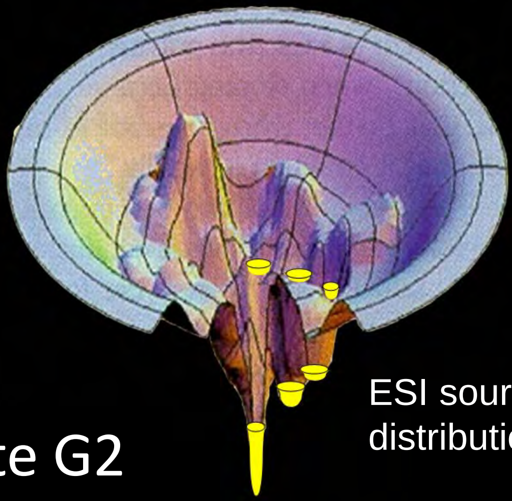
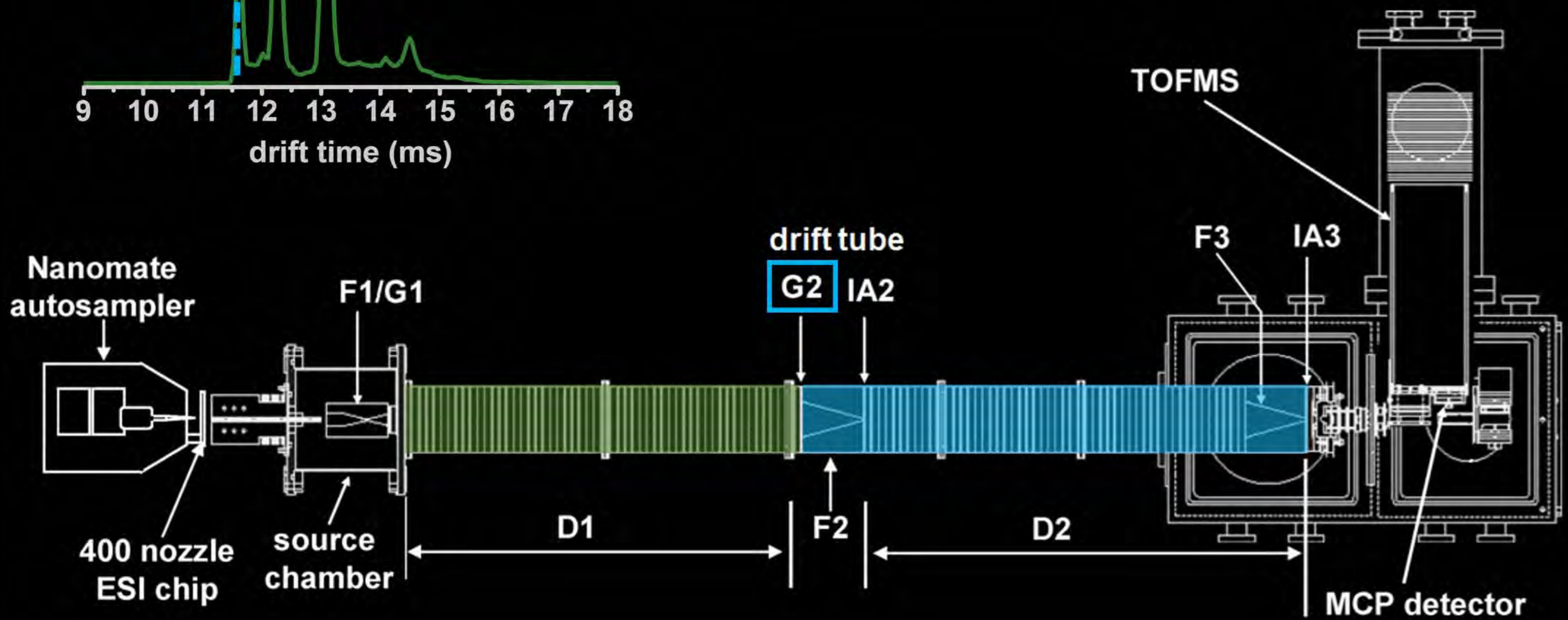


Apply delay pulse at ion gate G2

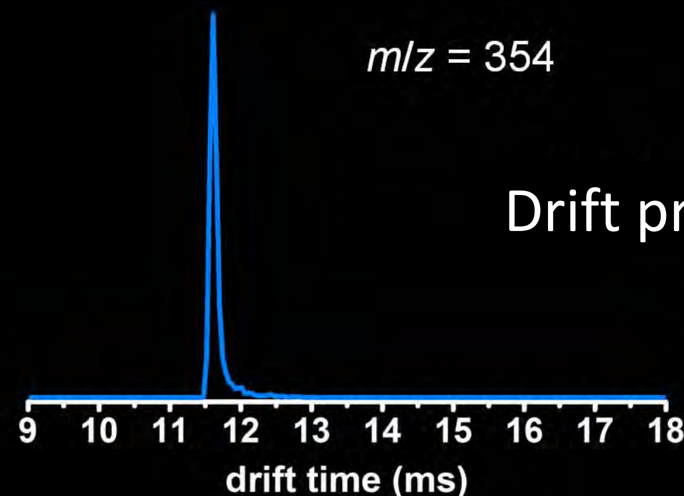


ESI source distribution

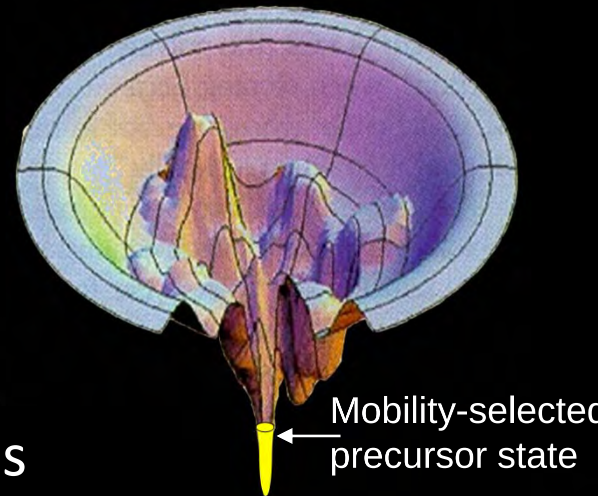


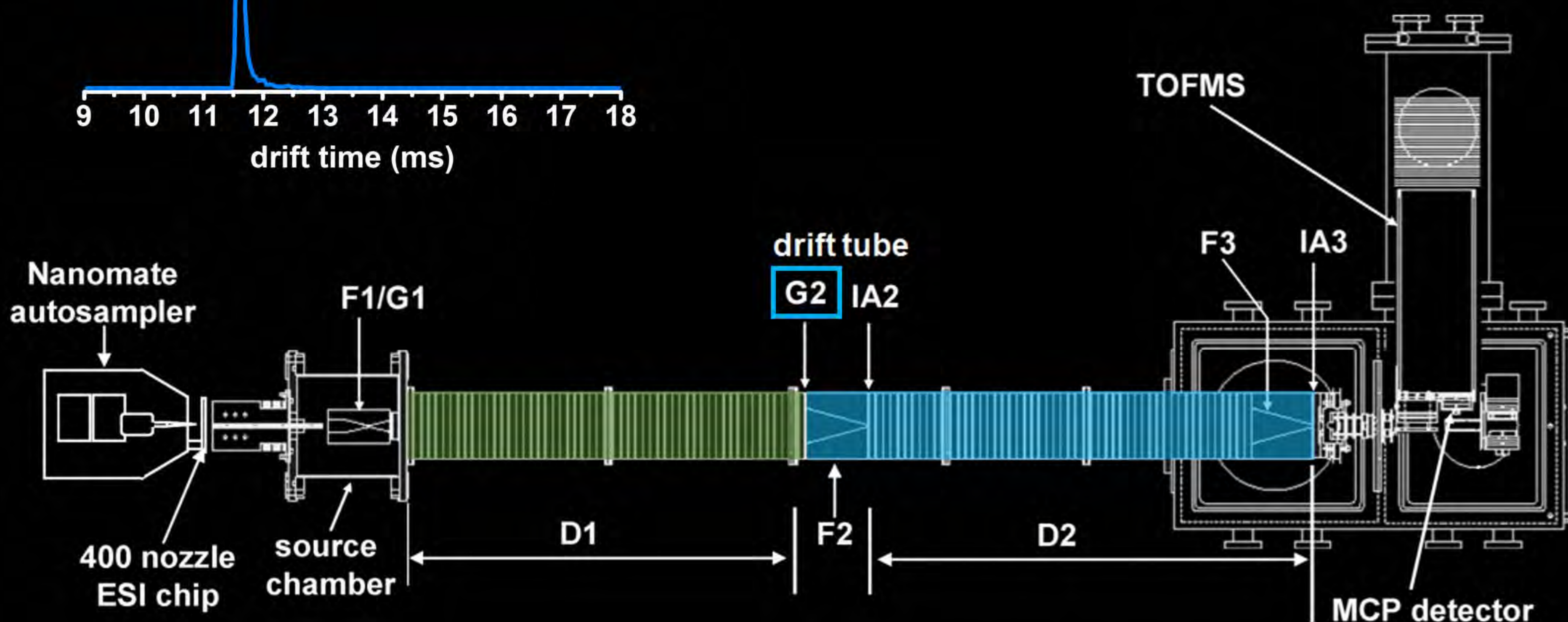
Surface modified from: Dill, K.A.; Chan, H. S. *Nature Structural Biology* **1997**, *4*, 10.

IMS-IMS-MS



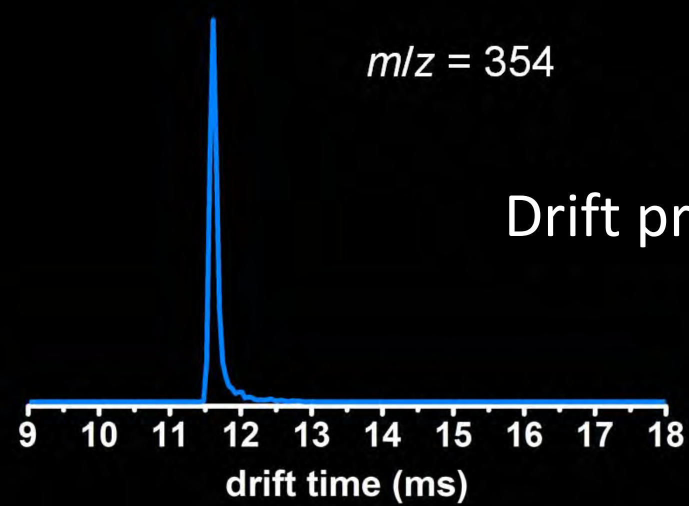
Drift profile of selected ions



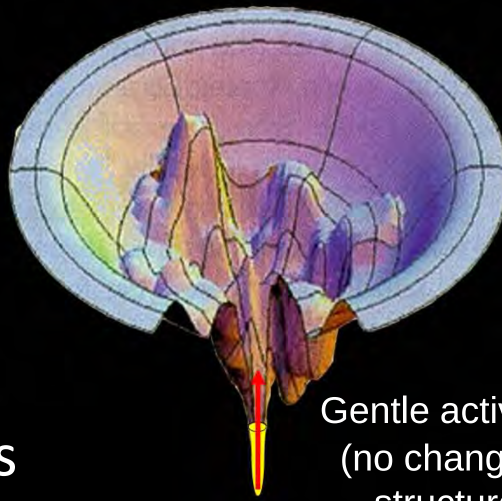


Surface modified from: Dill, K.A.; Chan, H. S. *Nature Structural Biology* **1997**, *4*, 10.

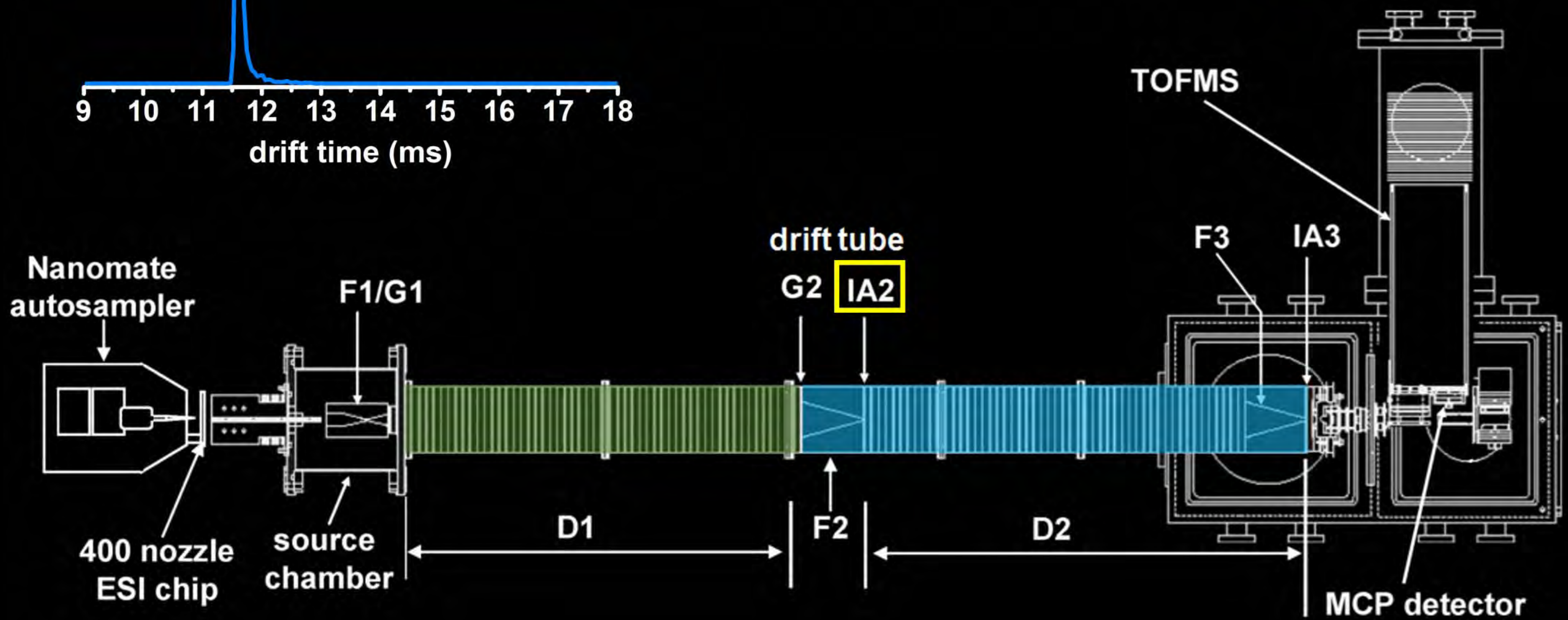
IMS-IMS-MS



Drift profile of selected ions



Gentle activation (no change in structure)



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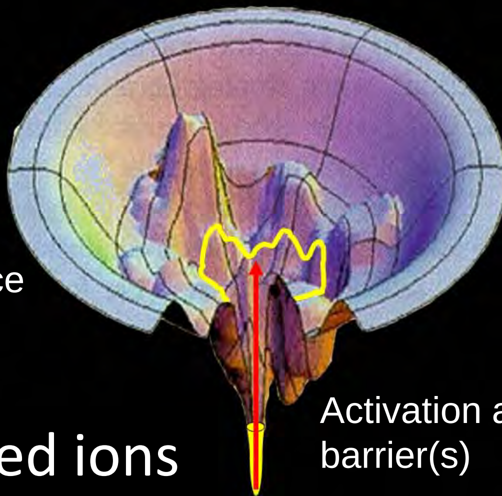
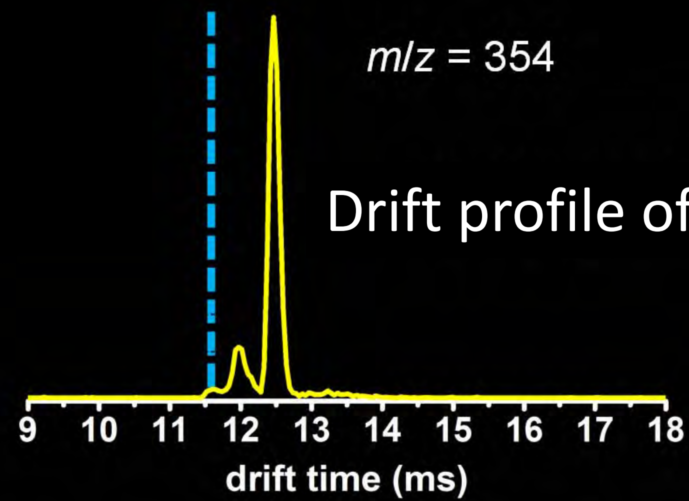
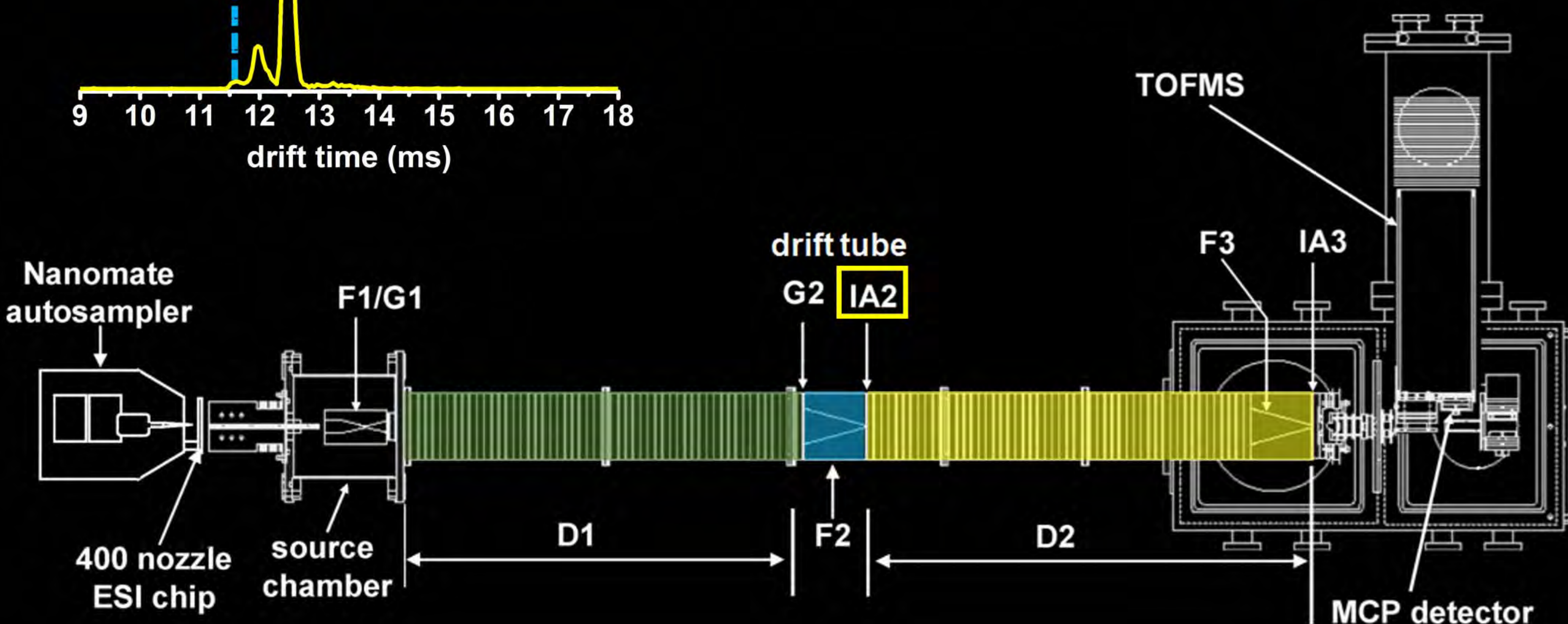


Drift profile of collisionally-activated ions





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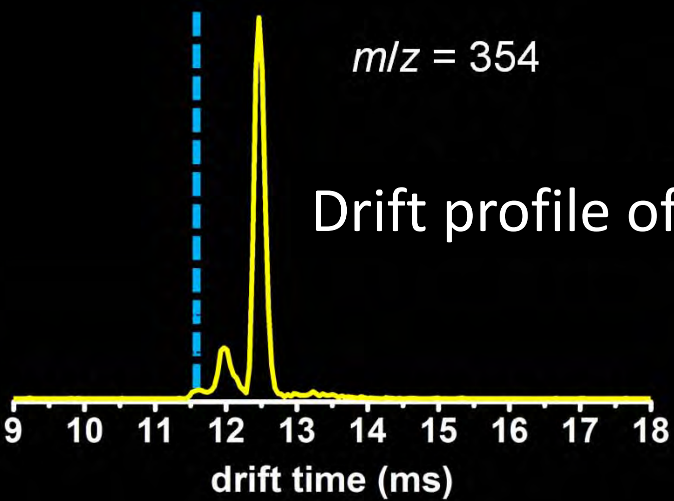
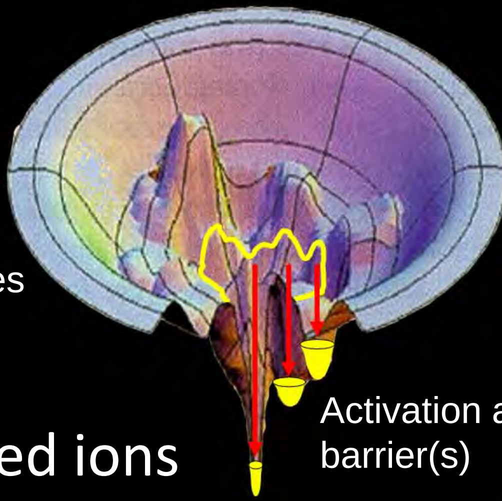
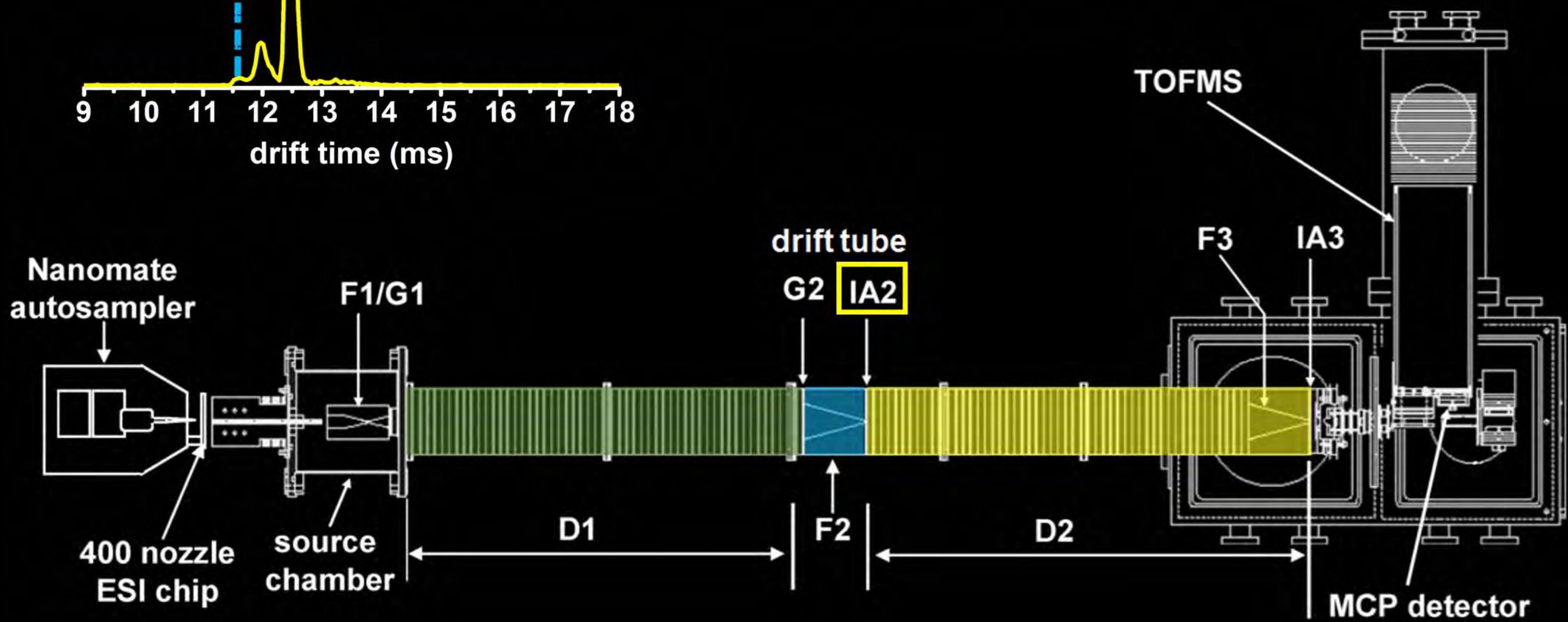
IMS-IMS-MS



Create new population of states



Drift profile of collisionally-activated ions



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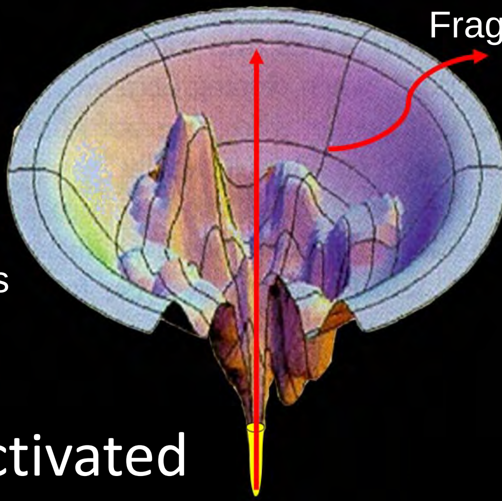
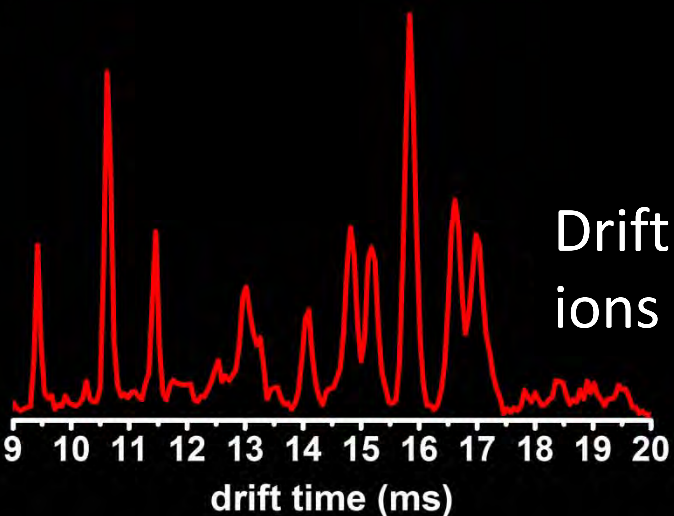
IMS-IMS-MS

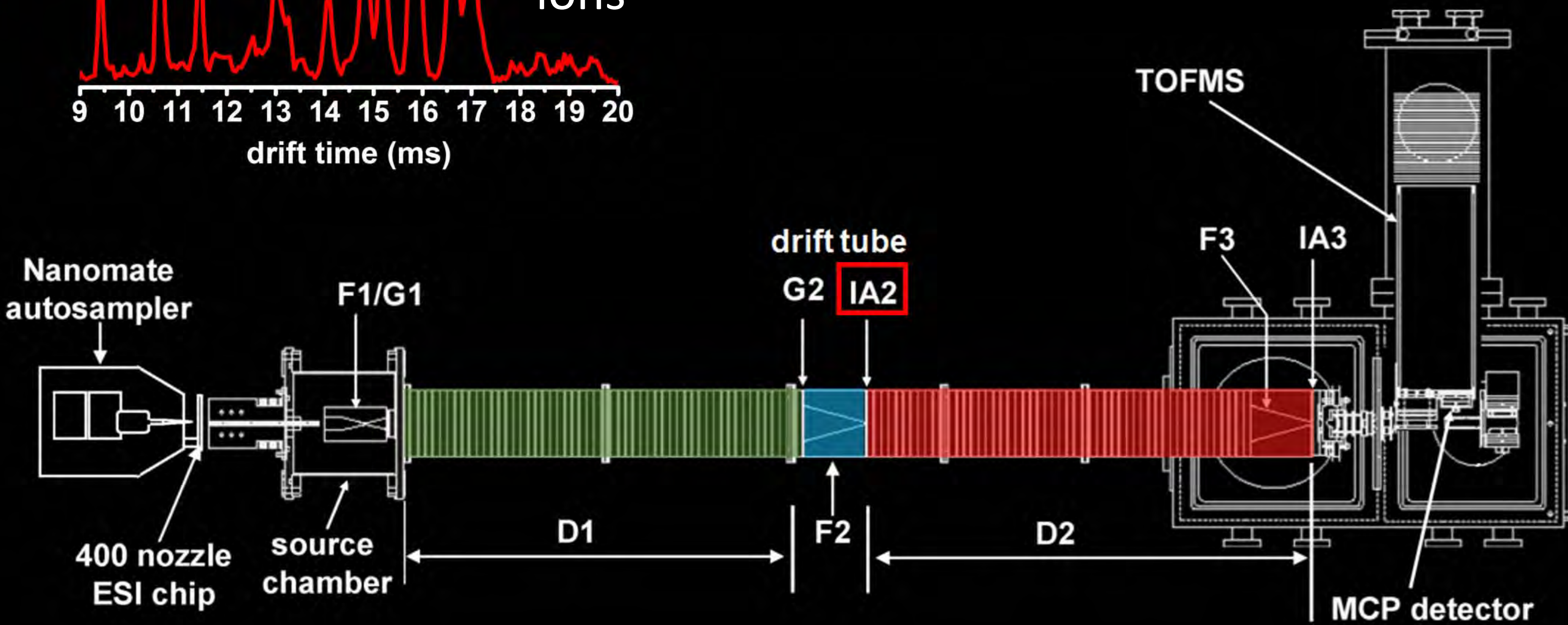


High activation voltages

→ Dissociation

Drift profile of harshly-activated ions





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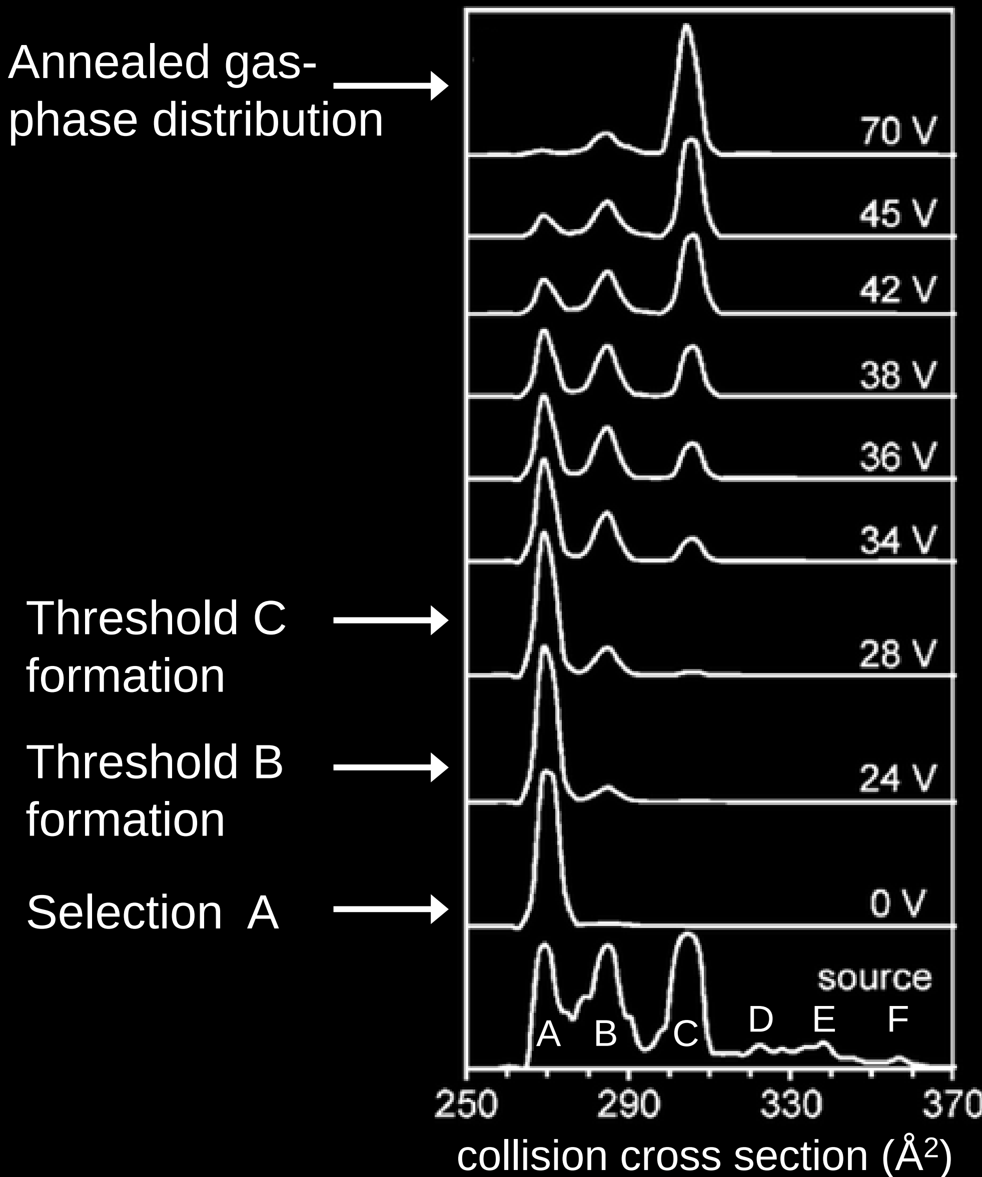
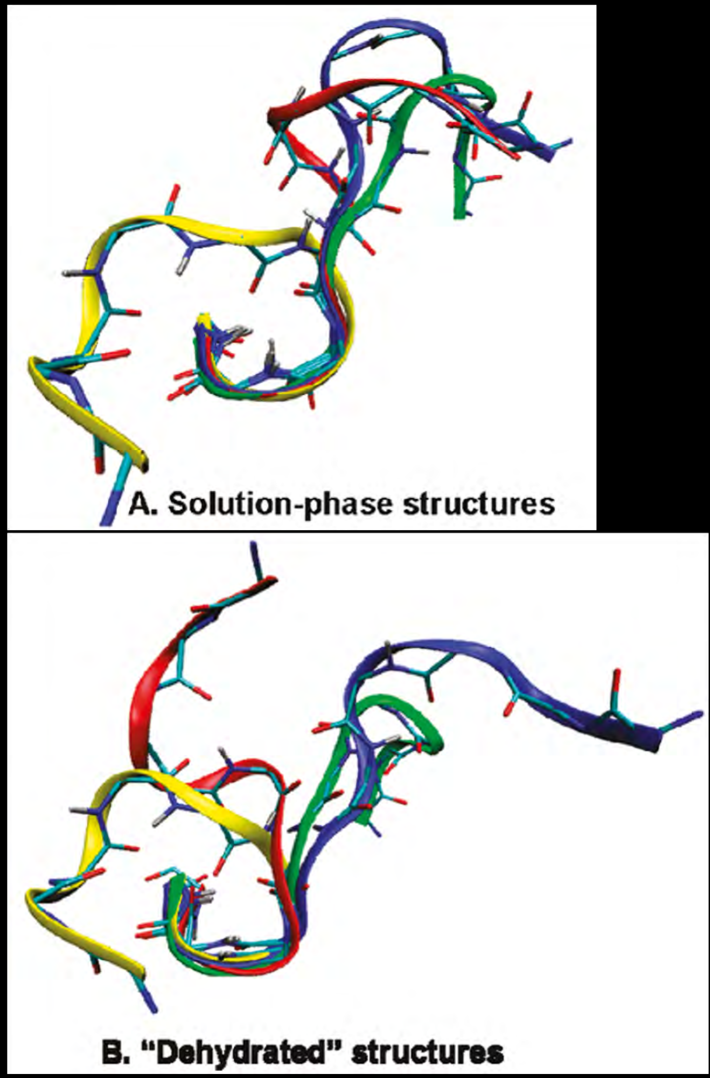
Voltage-Resolved Selection and Activation of Conformer A

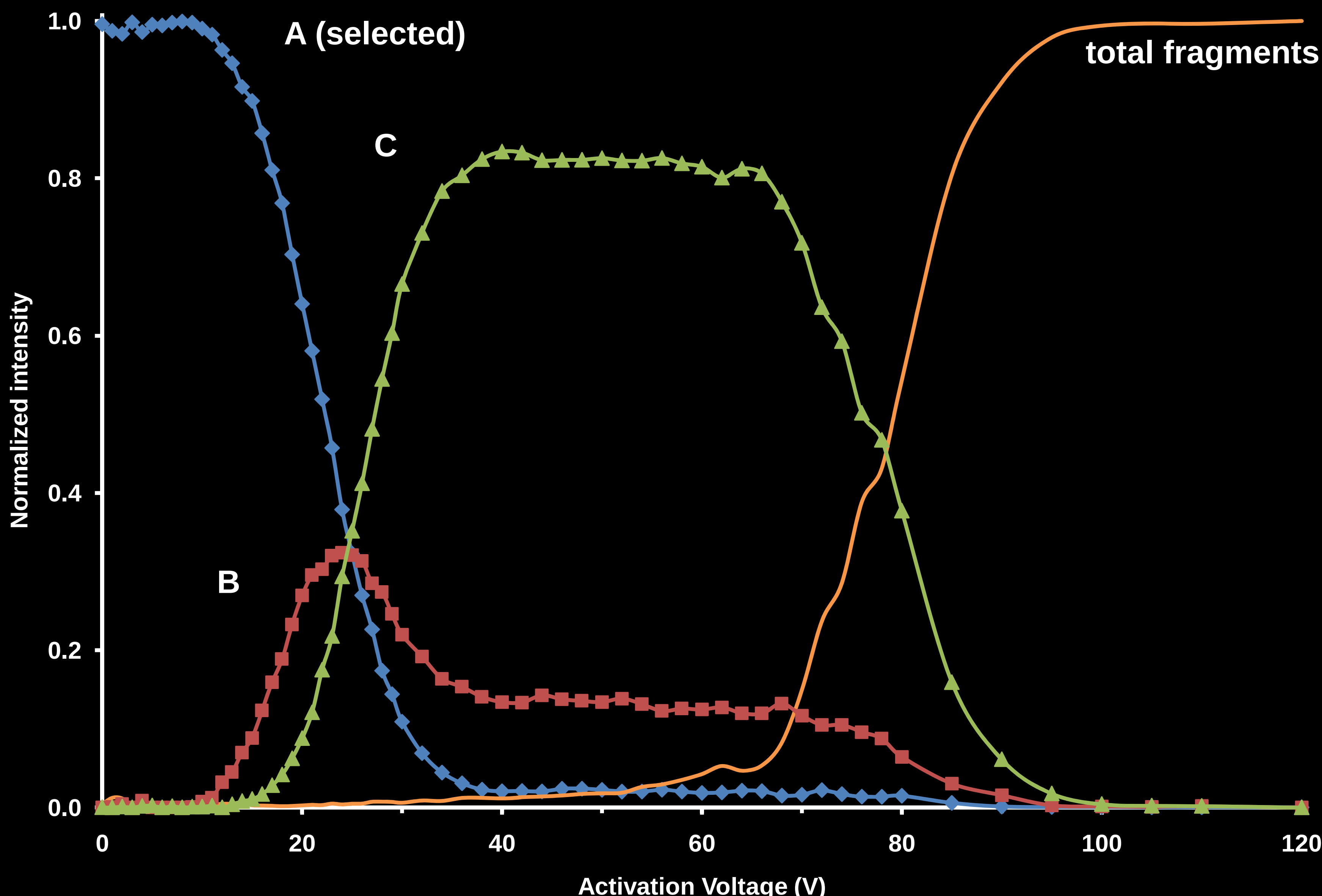




Pierson, N.A.; Valentine, S.J.; Clemmer, D.E. *J. Phys. Chem. B* **2010**, *114*, 7777–7783

Pierson, N.A.; Chen, L.; Valentine, S.J.; Russel, D.H.; Clemmer, D.E. *J. Am. Chem. Soc.* **2011**, *133*, 13810-13813

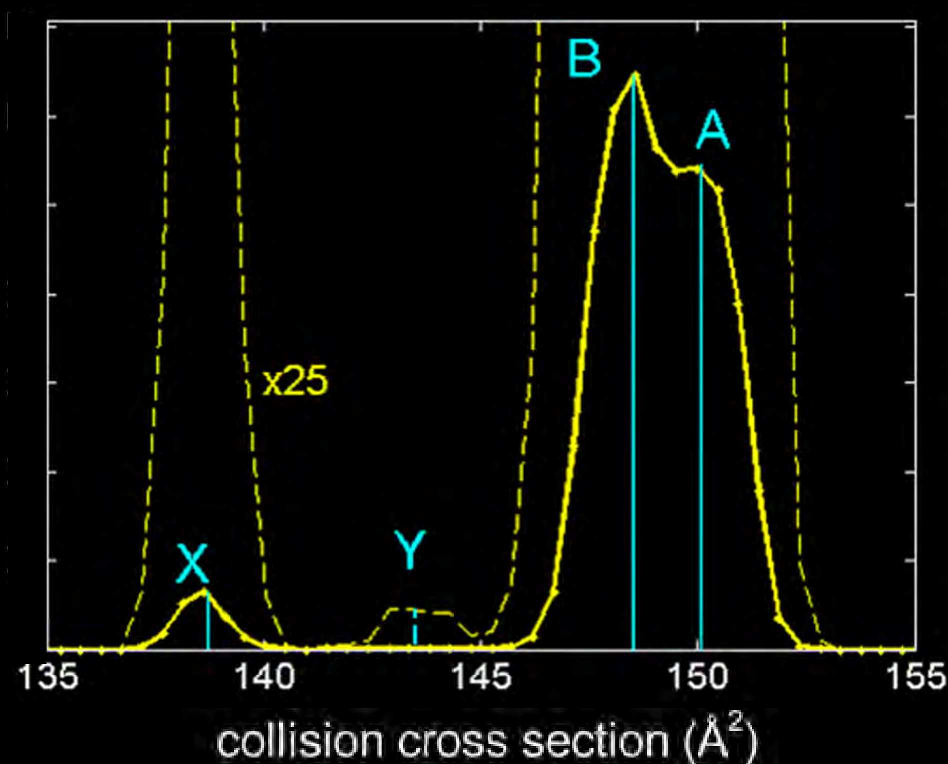
Voltage-Resolved Selection and Activation of Conformer A



Pierson, N.A.; Valentine, S.J.; Clemmer, D.E. *J. Phys. Chem. B* **2010**, *114*, 7777–7783

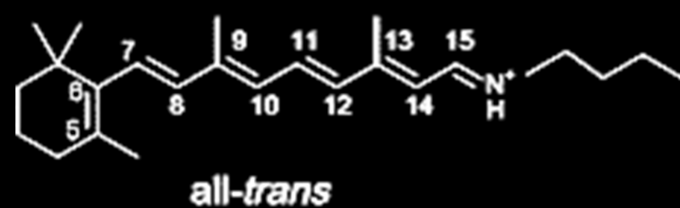
IMS-MS: $[RPSB+H]^+$

Retinal: $[RPSB+H]^+$
~340 amu



Peak A

- largest measured ccs
- calculated ccs of the *trans*-RPSB within 5%



Dilger J.M.; Musbat L.; Sheves M.; Bochenkova A.B.; Clemmer D.E.; Toker Y. *Ang. Chemie Int. Ed.* **2015**, *127*, 4830-4834

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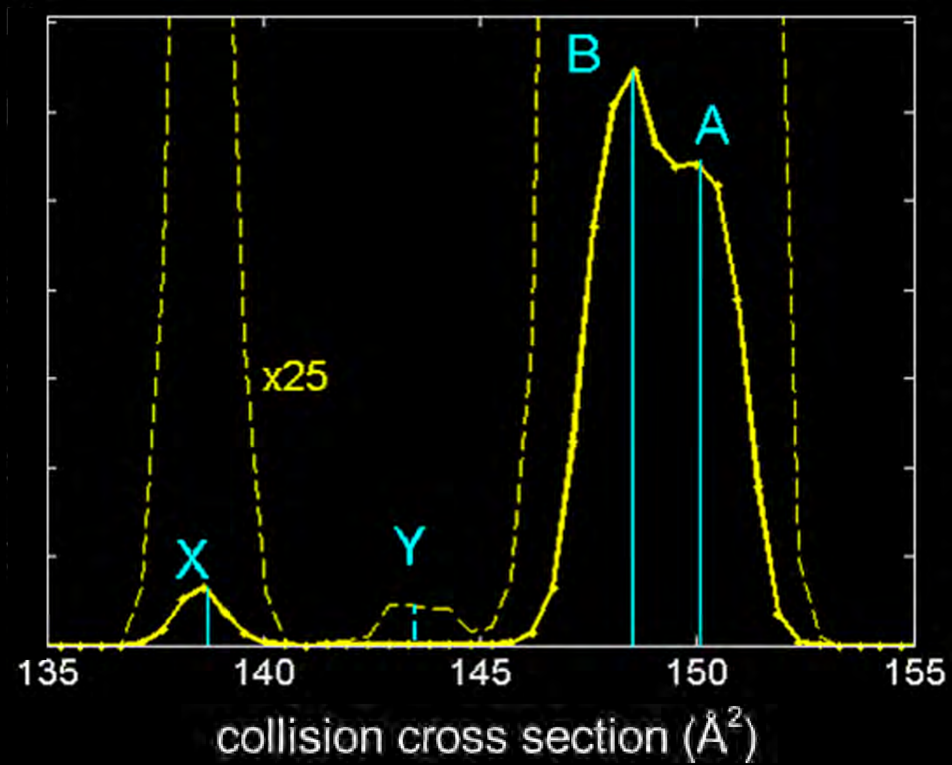
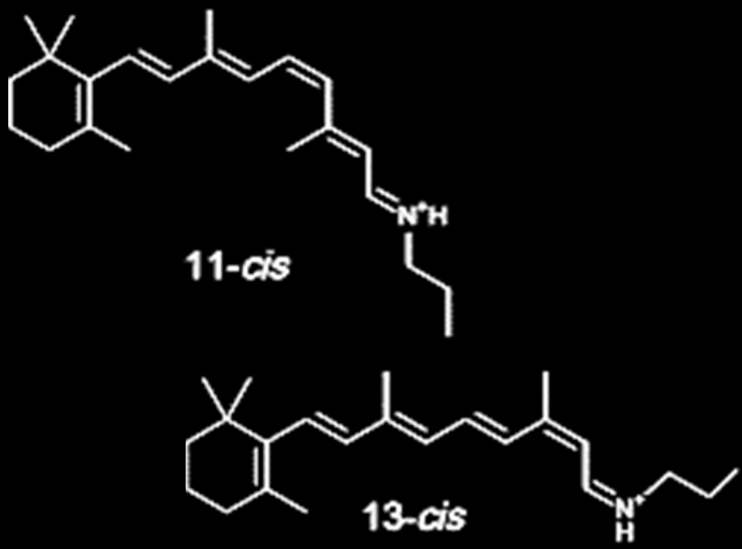
~340 amu



Peak B

- most abundant peak in the distribution
- calculated ccs agree with multiple *cis*-RPSB geometries



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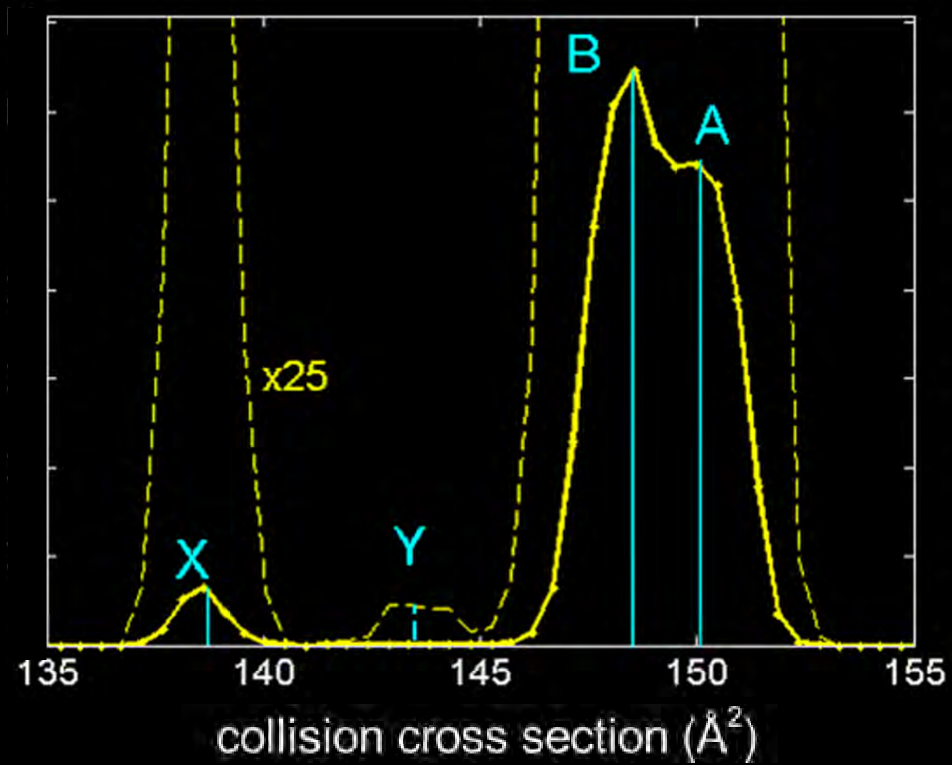
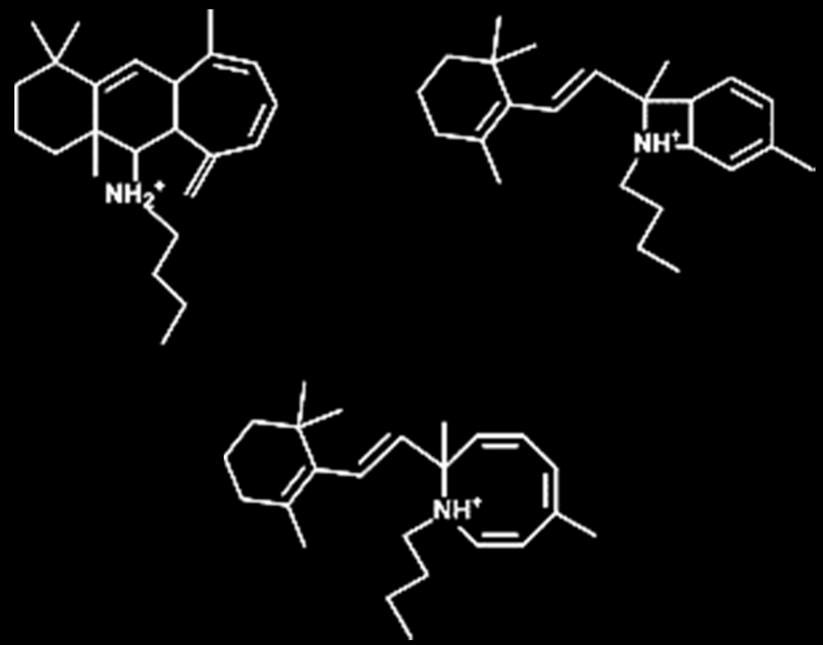


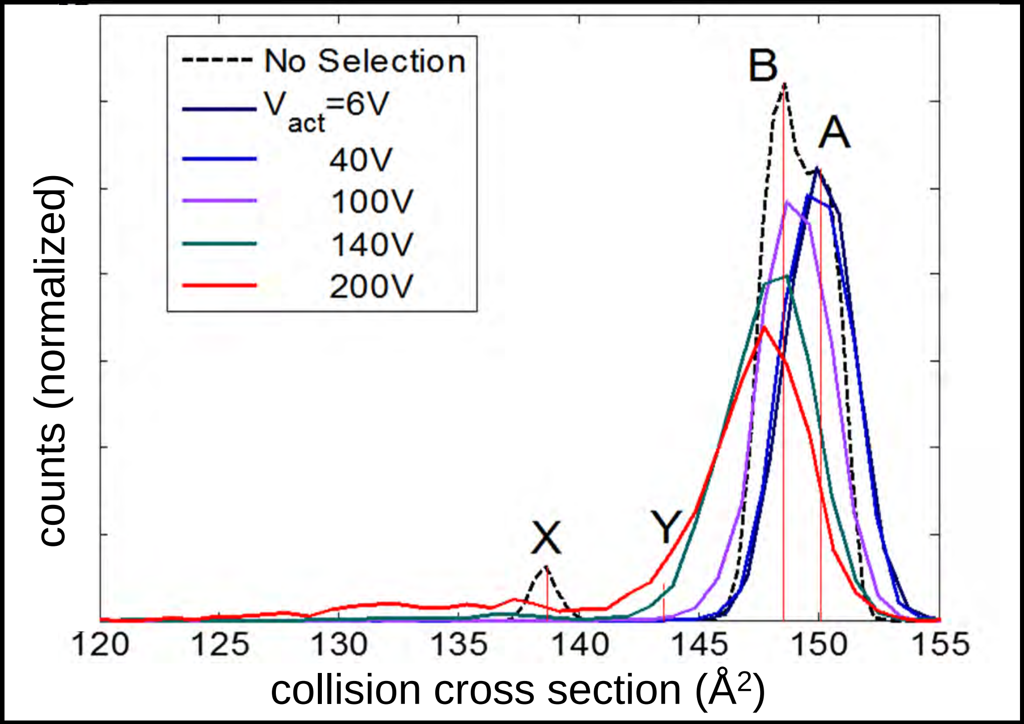
Peaks X & Y

- multiple-*cis* bonds
- potentially cyclized



Dilger J.M.; Musbat L.; Sheves M.; Bochenkova A.B.; Clemmer D.E.; Toker Y. *Ang. Chemie Int. Ed.* **2015**, *127*, 4830-4834

IMS-IMS-MS: Peak A of $[RPSB+H]^+$

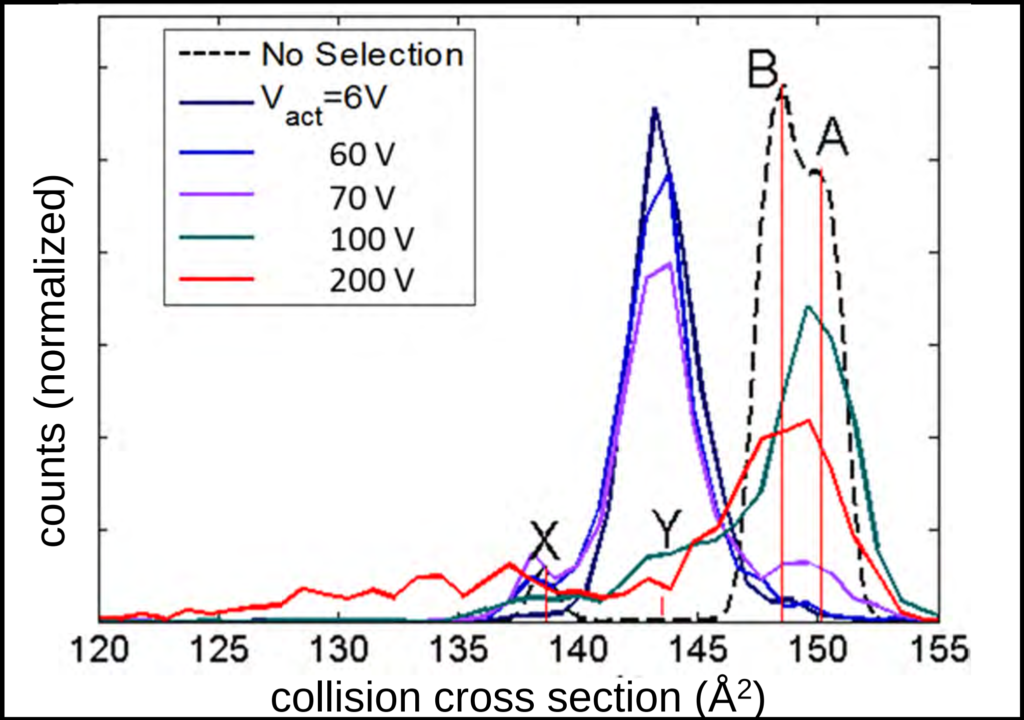


Activations were scanned in 10 V increments

Annealed gas-phase distribution shows a broad peak centered around Peak B

Dilger J.M.; Musbat L.; Sheves M.; Bochenkova A.B.; Clemmer D.E.; Toker Y. *Ang. Chemie Int. Ed.* **2015**, *127*, 4830-4834

IMS-IMS-MS: Peak Y of $[RPSB+H]^+$



Activations were scanned in 10 V increments

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Dilger J.M.; Musbat L.; Sheves M.; Bochenkova A.B.; Clemmer D.E.; Toker Y. *Ang. Chemie Int. Ed.* **2015**, *127*, 4830-4834

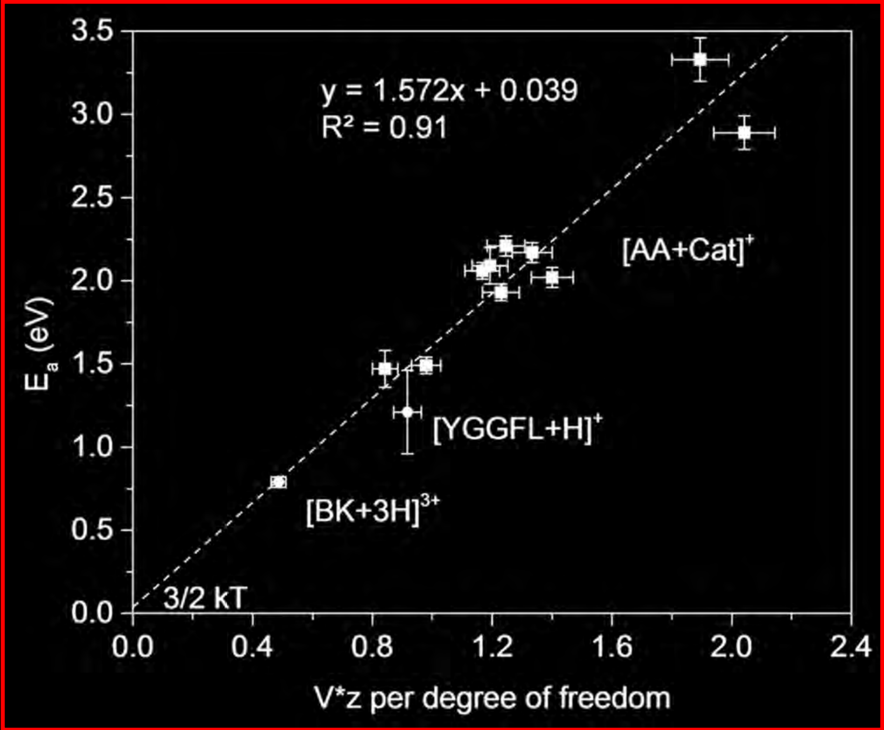
Measuring Activation Energies by IMS–IMS

We observe pre-dissociation state-to-state transitions, but at what energies?

- Use of an external IA2 voltage-to-eV calibration by measuring well-characterized dissociation events

“Thermometer ion” systems:

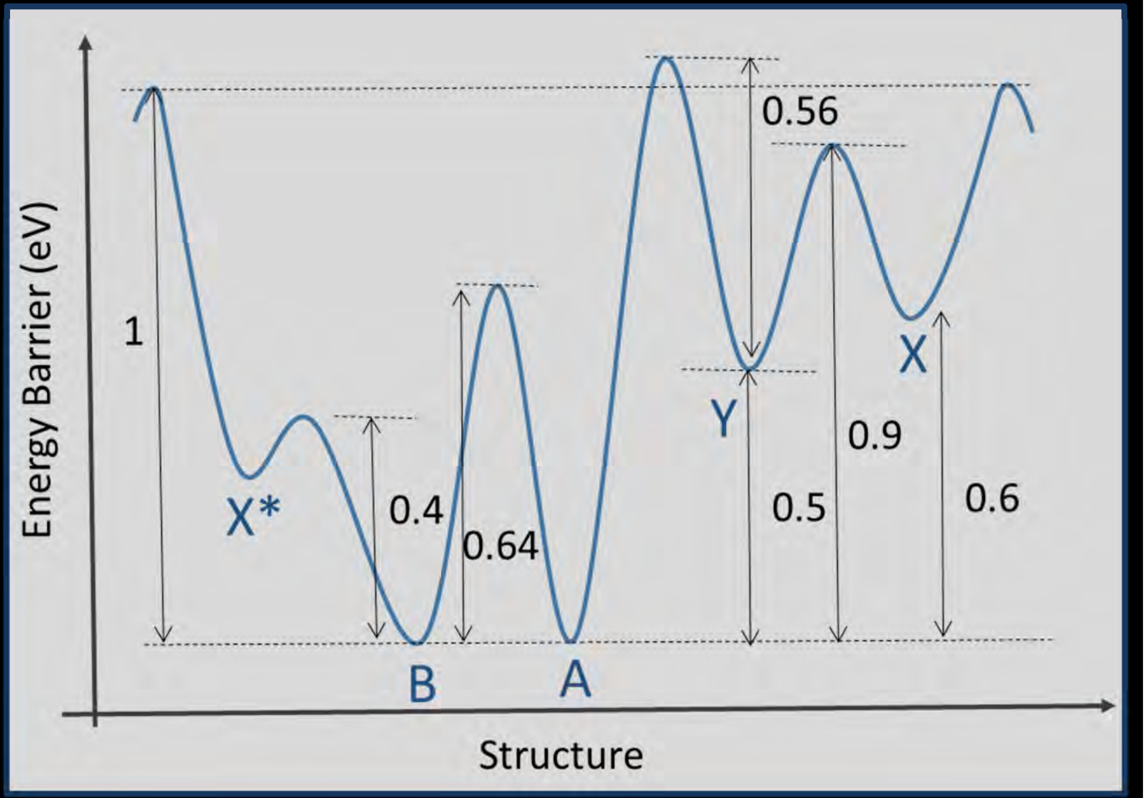
Ion	E_a, eV	IA2 Voltage · z
$[Pro+K]^+$	1.49 (±0.05)	47 (±2.4)
$[Met+K]^+$	1.47 (±0.11)	48 (±2.4)
$[Asp+Na]^+$	2.02 (±0.06)	63 (±3.2)
$[Asn+Na]^+$	2.17 (±0.06)	64 (±3.2)
$[Glu+Na]^+$	2.06 (±0.05)	63 (±3.2)
$[Gln+Na]^+$	2.21 (±0.06)	71 (±3.6)
$[Pro+Na]^+$	1.93 (±0.05)	59 (±3.0)
$[Met Na]^+$	2.09 (±0.11)	68 (±3.4)
$[Pro Li]^+$	2.89 (±0.10)	98 (±4.9)
$[Met Li]^+$	3.33 (±0.13)	108 (±5.4)
$[YGGFL+H]^+$	1.21 (±0.25)	209 (±10.5)
$[BK+3H]^{3+}$	0.79 (±0.03)	219 (±3.7)



Pierson, N.A.; Clemmer, D.E. *Int. J. Mass Spectrom.* **2015**, *377*, 646–654

Measuring Activation Energies by IMS–IMS

Process	Barrier Energy (eV)
A→X	0.9 (±0.05)
B→X	0.4 (±0.05)
Y→A	0.56 (±0.05)
Y→B	0.56 (±0.05)
Y→X	0.5 (±0.05)
X→A	0.5 (±0.05)
X→B	0.5 (±0.05)
A→B	0.64 (±0.05)
A→B	0.64 (±0.05)
A→*frag*	1.0 (±0.05)
B→*frag*	1.0 (±0.05)
Y→*frag*	0.5 (±0.05)
X→*frag*	0.4 (±0.05)



Dilger J.M.; Musbat L.; Sheves M.; Bochenkova A.B.; Clemmer D.E.; Toker Y. *Ang. Chemie Int. Ed.* **2015**, *127*, 4830-4834

Measuring Activation Energies by IMS–IMS

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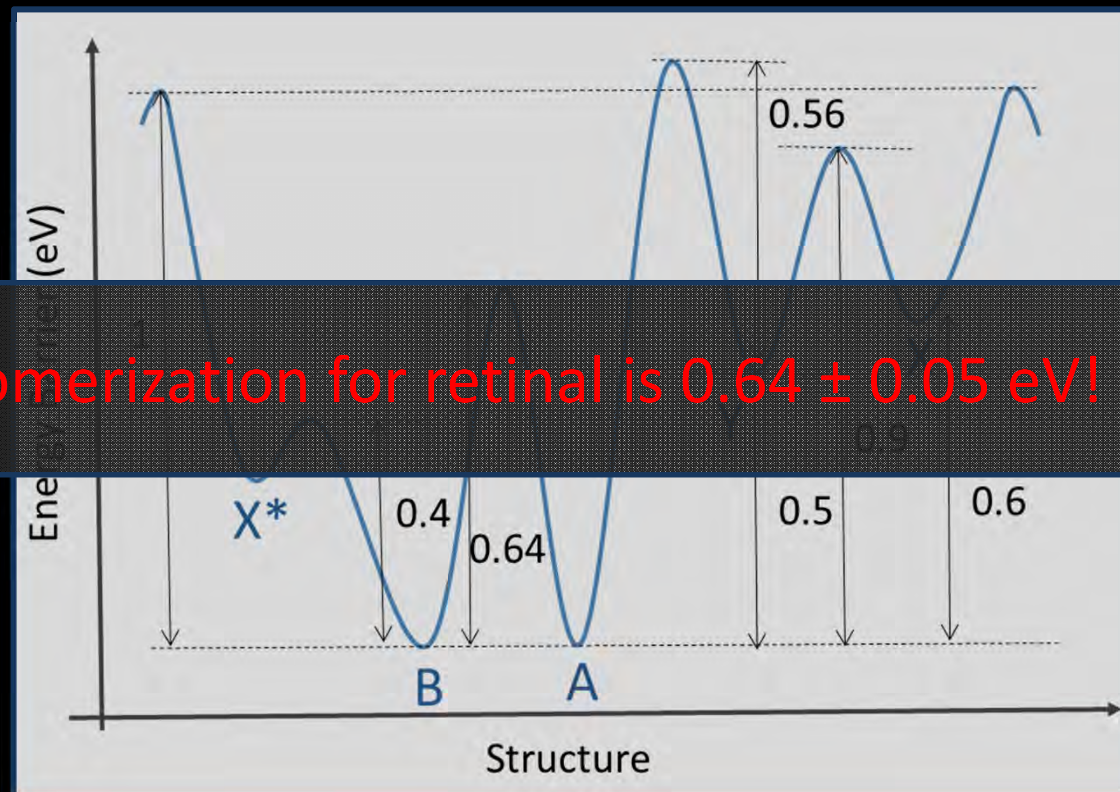
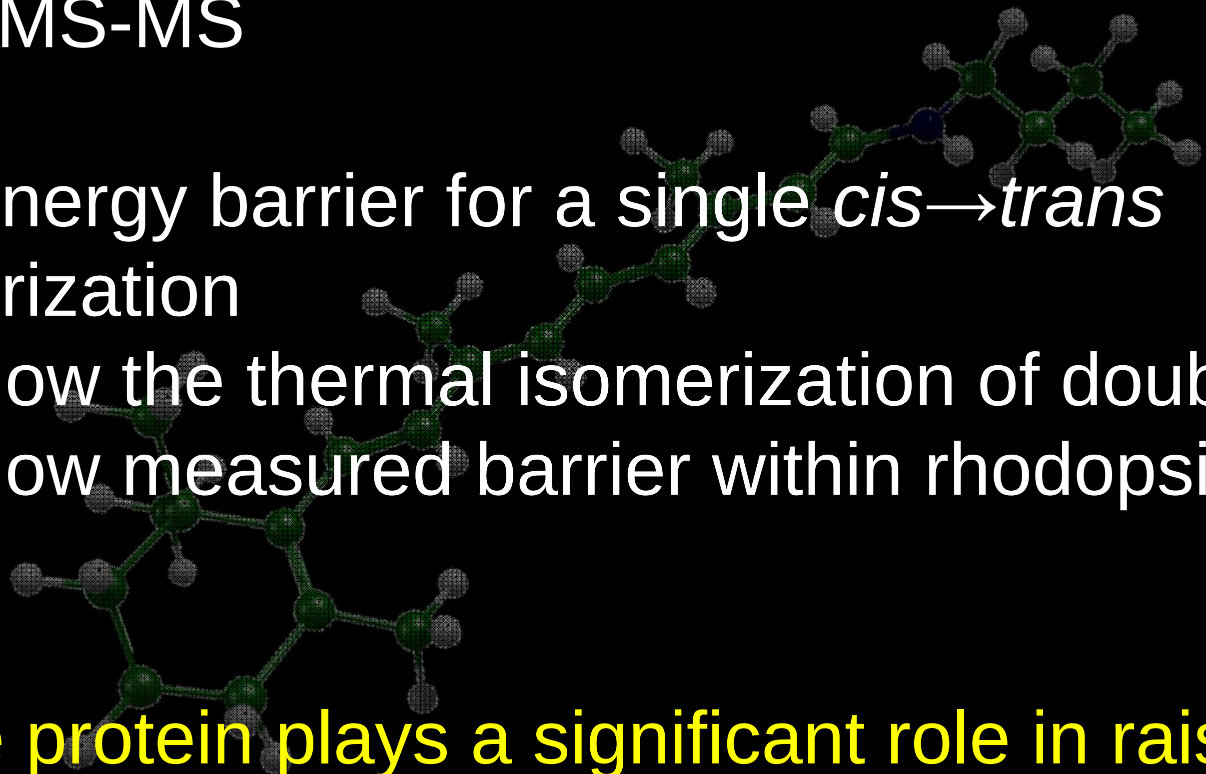


Thus, a ***single*** *cis-trans* isomerization for retinal is 0.64 ± 0.05 eV!

Dilger J.M.; Musbat L.; Sheves M.; Bochenkova A.B.; Clemmer D.E.; Toker Y. *Ang. Chemie Int. Ed.* **2015**, *127*, 4830-4834

Summary

- Isomerization barriers of isolated $[RPSB+H]^+$ via IMS–IMS-MS
- Low energy barrier for a single *cis*→*trans* isomerization
  - Below the thermal isomerization of double bonds
  - Below measured barrier within rhodopsin proteins



Thus, the protein plays a significant role in raising the energetic barrier for photoisomerization of retinal

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S. Brøndsted Nielsen

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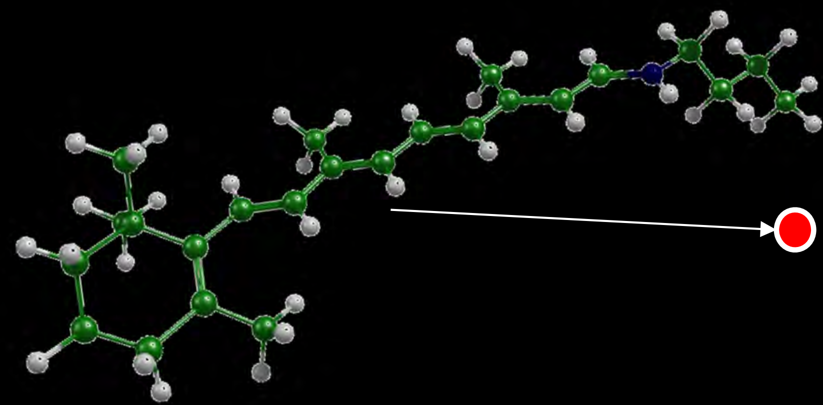
Mordechai Sheves

Funding:
NSWC Crane NISE/ 219
Indiana University METACyt

Backup Slides

Ion Mobility Spectrometry (IMS)

Study the shape of a molecule through collisions with neutral atoms/molecules



- Neutral atoms are as small as the features we want to study

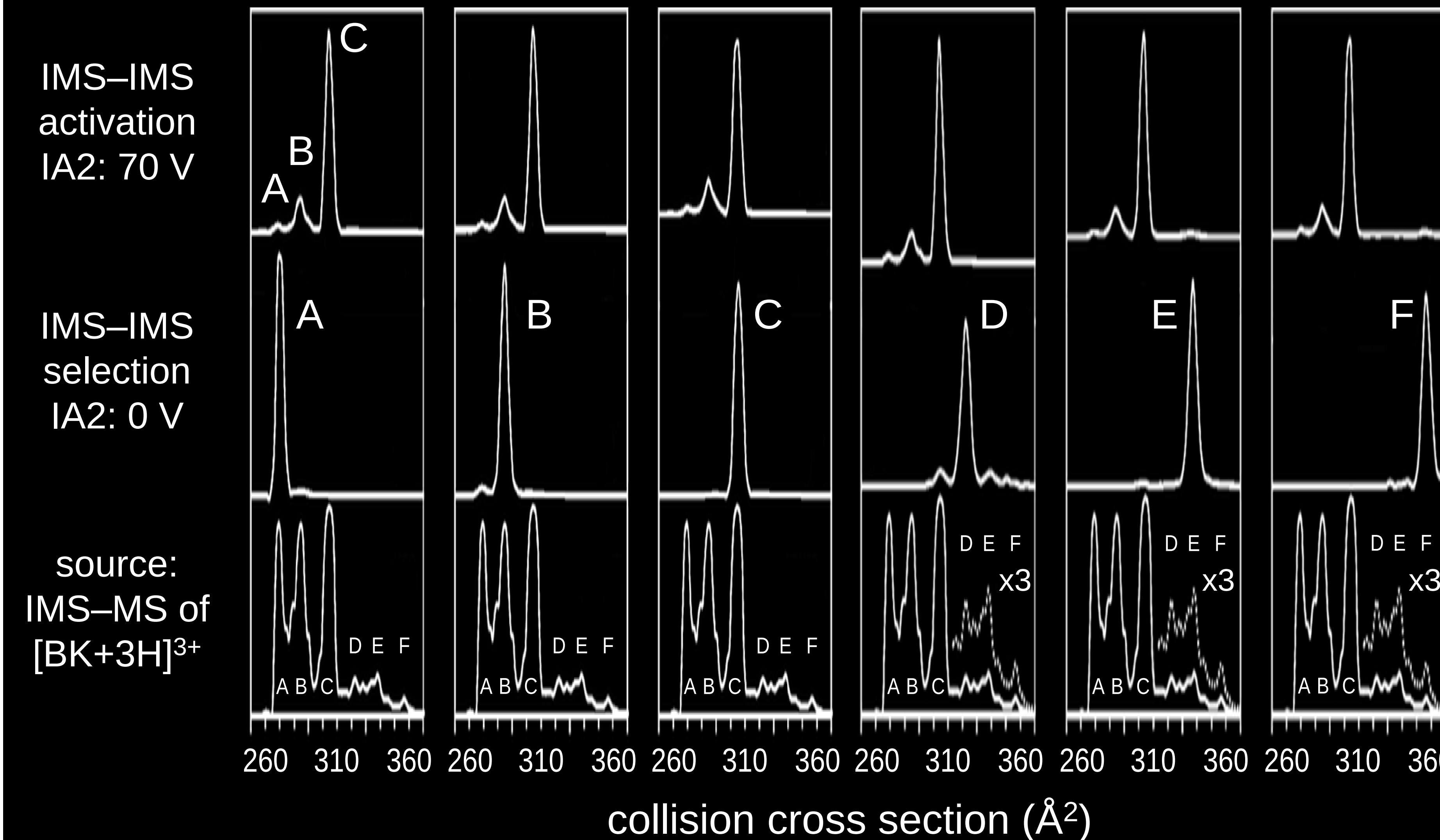
- Collision energy can be set to be very small

In order to study the structure

- And can also set to be large

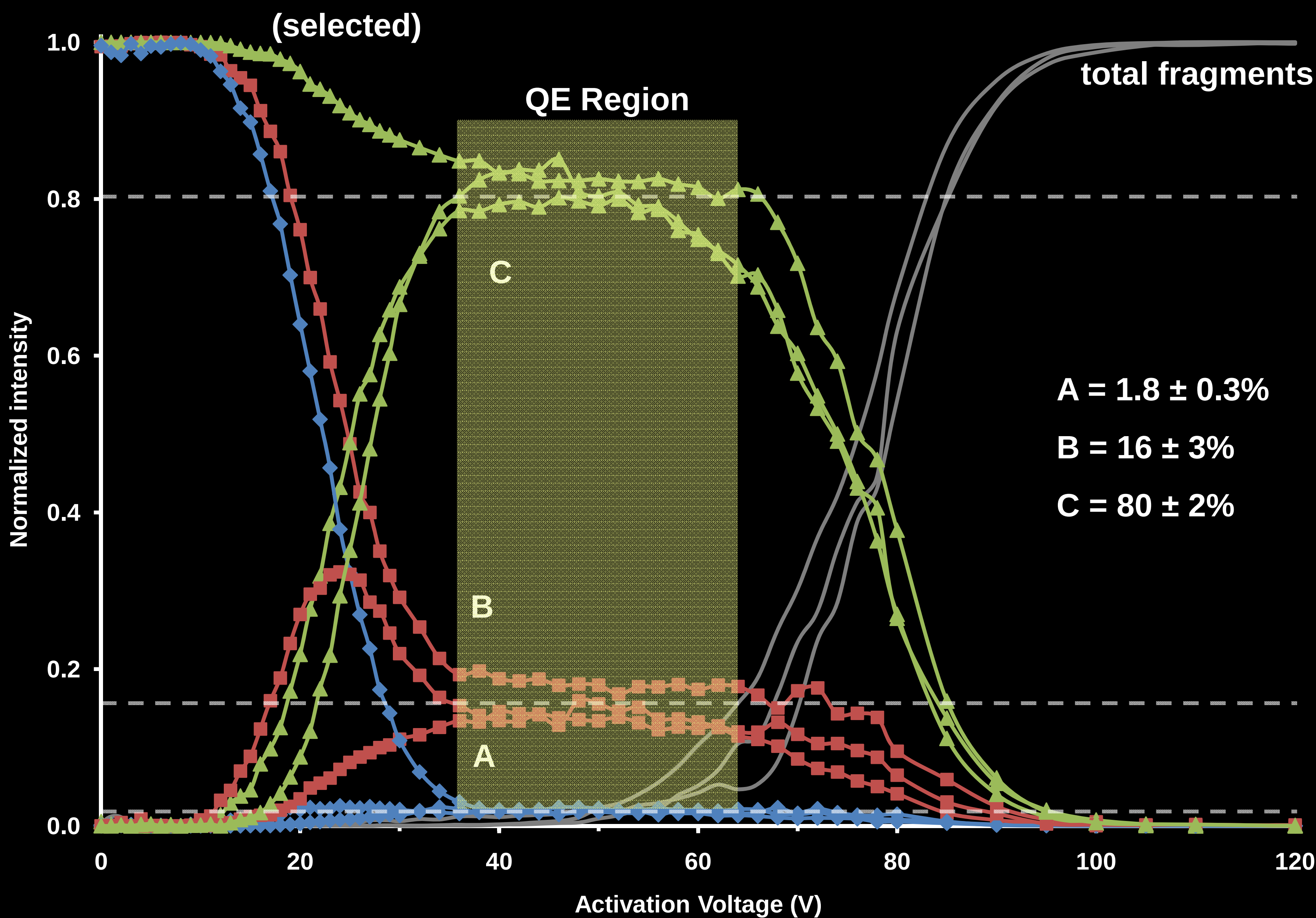
In order to heat the molecule, cause it to isomerize, and eventually break apart.

Gas-Phase Distributions of Bradykinin



Pierson, N.A.; Valentine, S.J.; Clemmer, D.E. *J. Phys. Chem. B* **2010**, *114*, 7777–7783

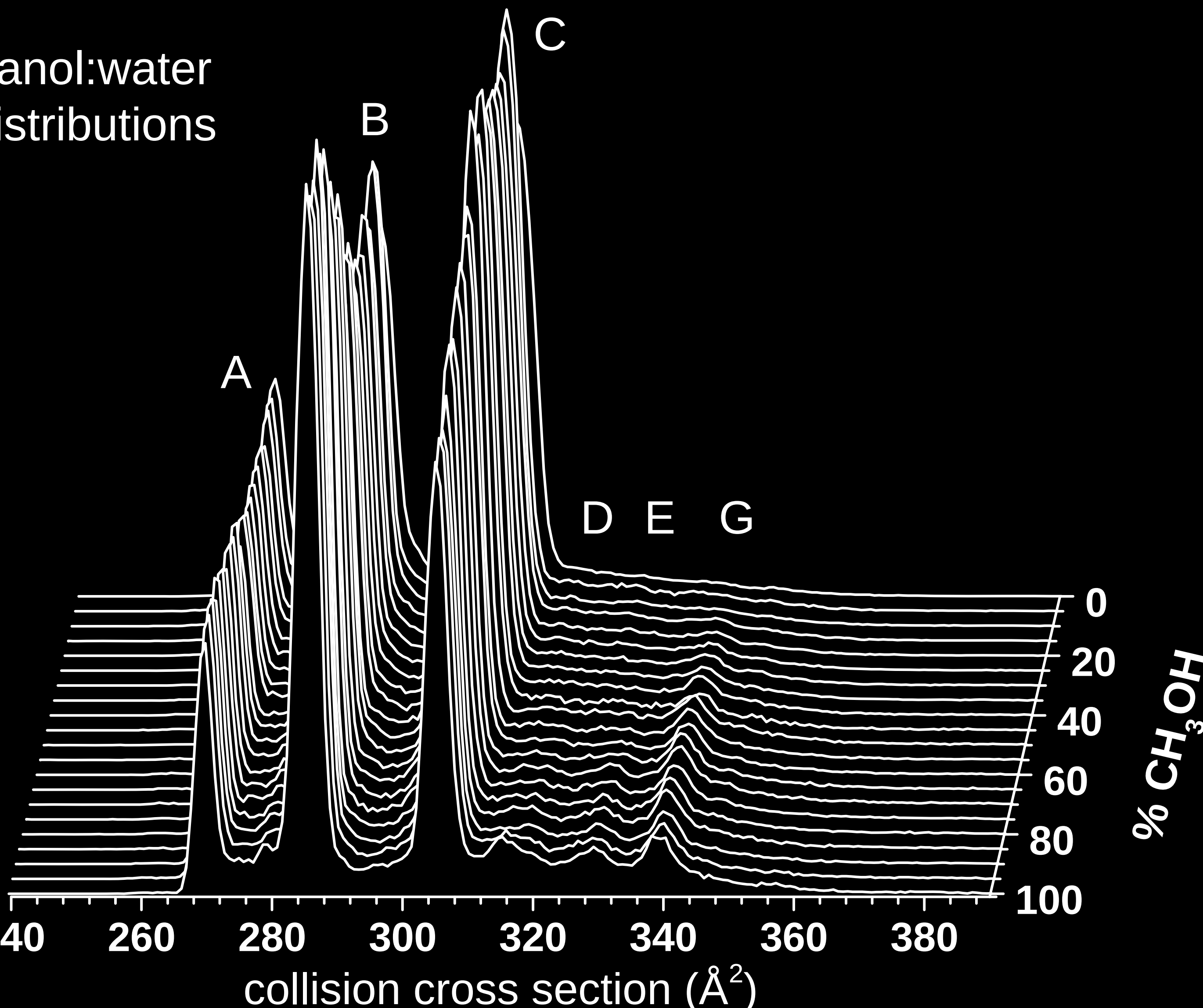
Selection and Activation of Conformers A, B, and C



Pierson, N.A.; Valentine, S.J.; Clemmer, D.E. *J. Phys. Chem. B* **2010**, *114*, 7777–7783

ESI Solution Studies

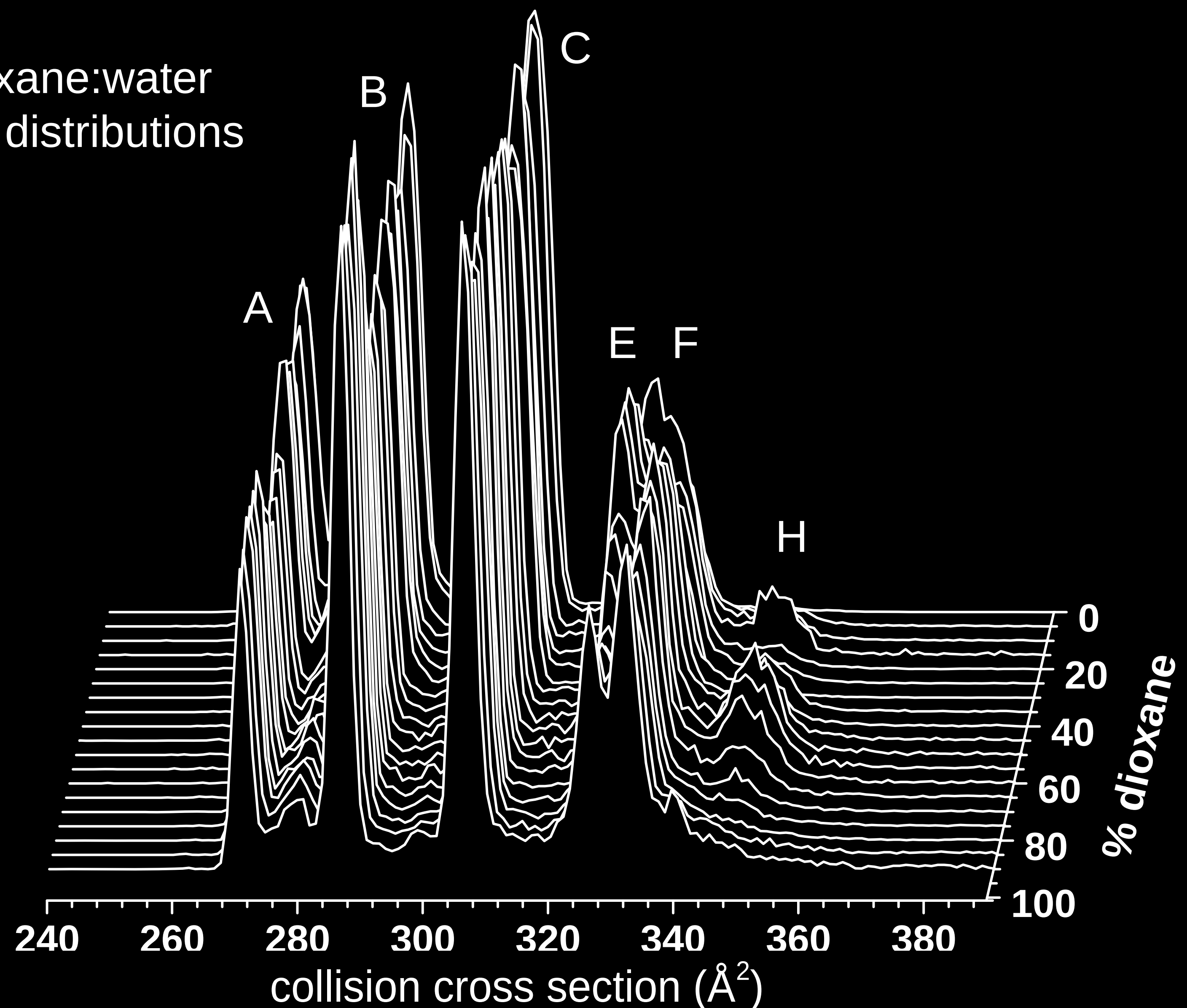
BK in methanol:water
$[M+3H]^{3+}$ distributions



Pierson, N.A.; Chen, L.; Valentine, S.J.; Russell, D.H.; Clemmer, D.E. *J. Am. Chem. Soc.* **2011**, *133*, 13810

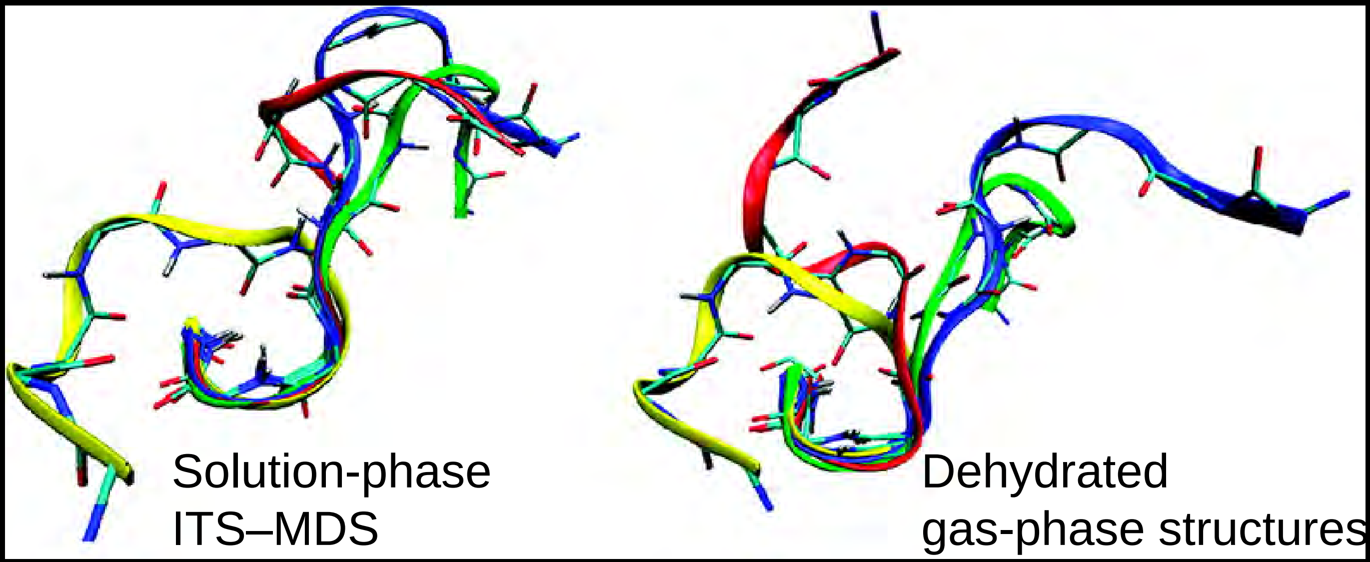
ESI Solution Studies

BK in dioxane:water
$[M+3H]^{3+}$ distributions



Pierson, N.A.; Chen, L.; Valentine, S.J.; Russell, D.H.; Clemmer, D.E. *J. Am. Chem. Soc.* **2011**, *133*, 13810

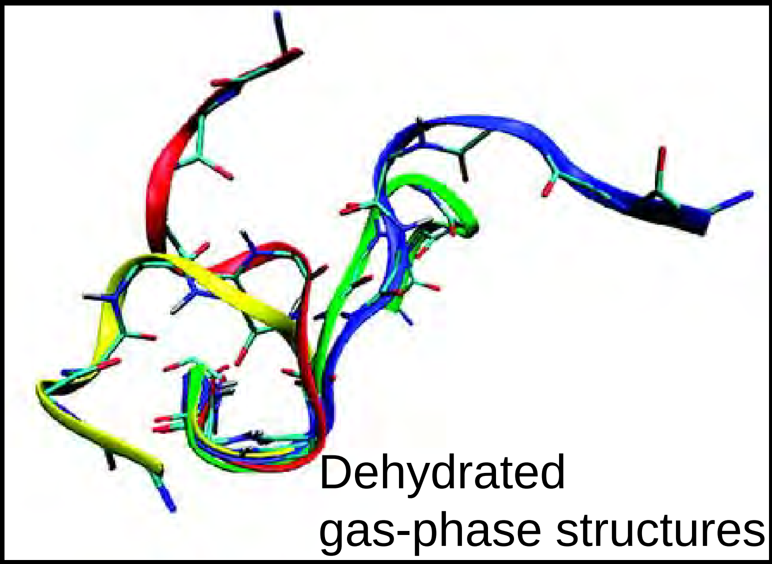
Findings from BK Solution Scans

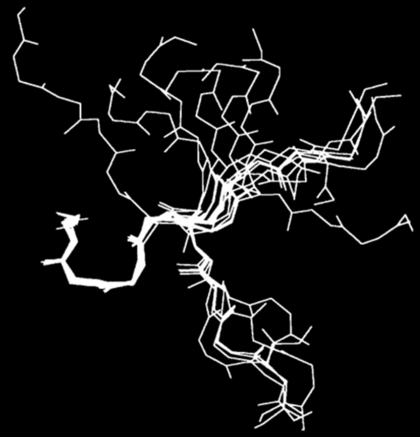


- Amino-terminal "unstructured" region consists of multiple, stable conformations
- Highly complementary to solution-phase characterization methods

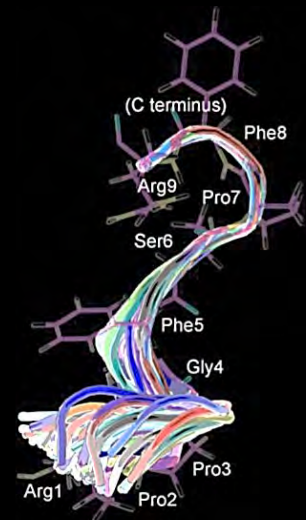
Pierson, N.A.; Chen, L.; Valentine, S.J.; Russell, D.H.; Clemmer, D.E. *J. Am. Chem. Soc.* **2011**, *133*, 13810

Findings from BK Solution Scans





Young, J. K.; Hicks, R. P. *Biopolymers* **1994**, *34*, 611–623



Lopez, J. J. et al. *Angew. Chem. Int. Ed.* **2008**, *47*, 1668 –1671

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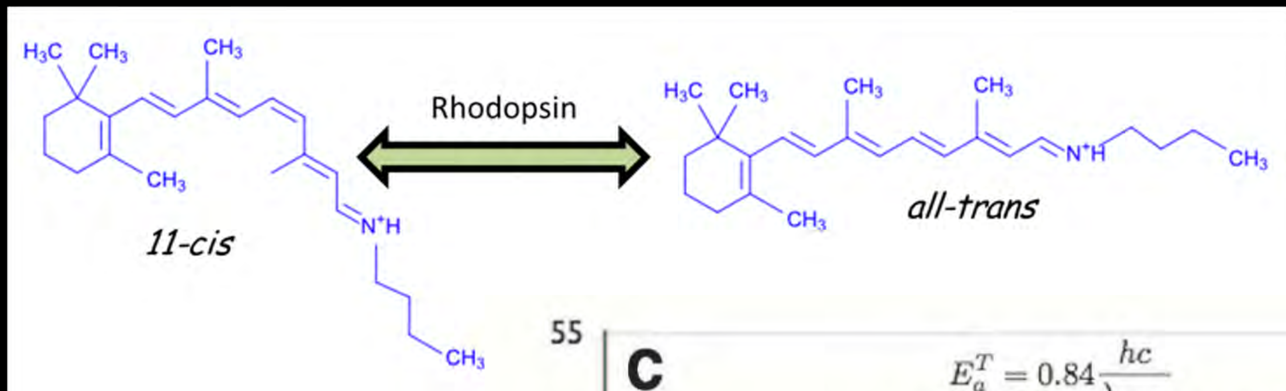
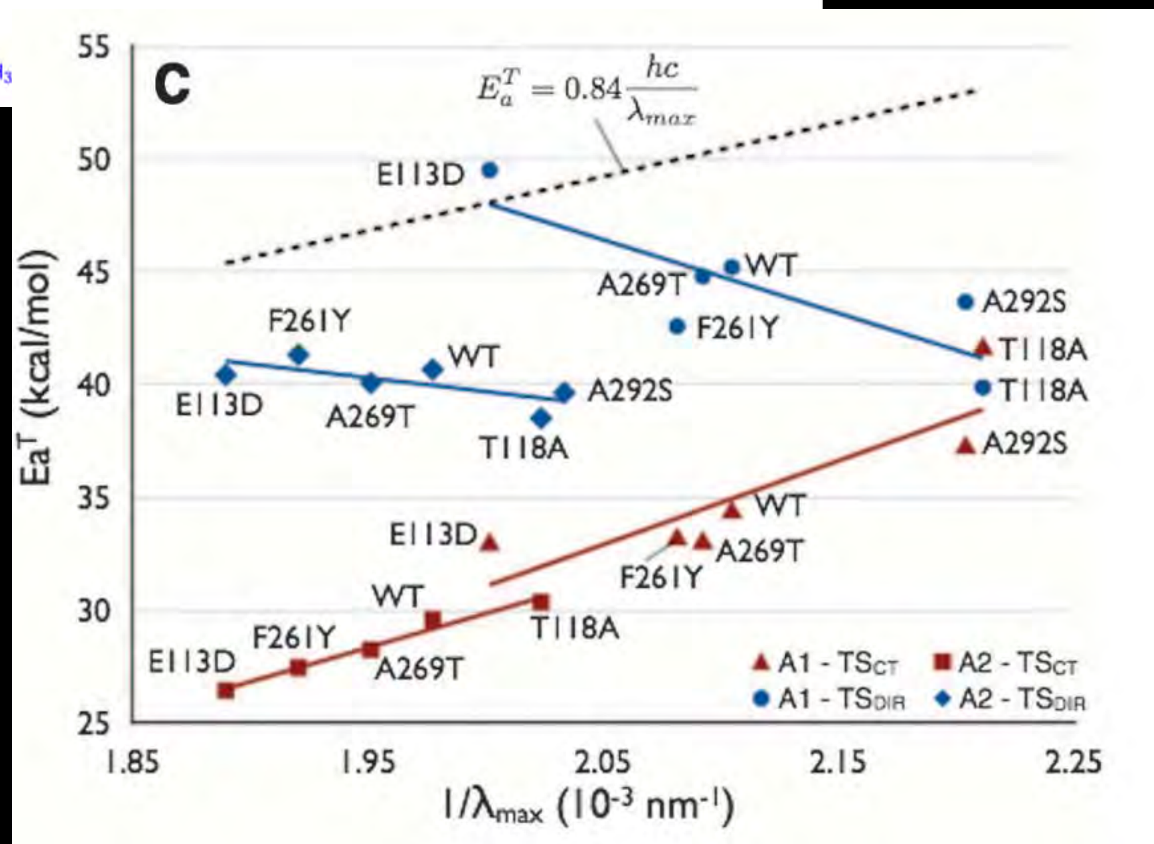
Pierson, N.A.; Chen, L.; Valentine, S.J.; Russell, D.H.; Clemmer, D.E. *J. Am. Chem. Soc.* **2011**, *133*, 13810

Barlow Correlation



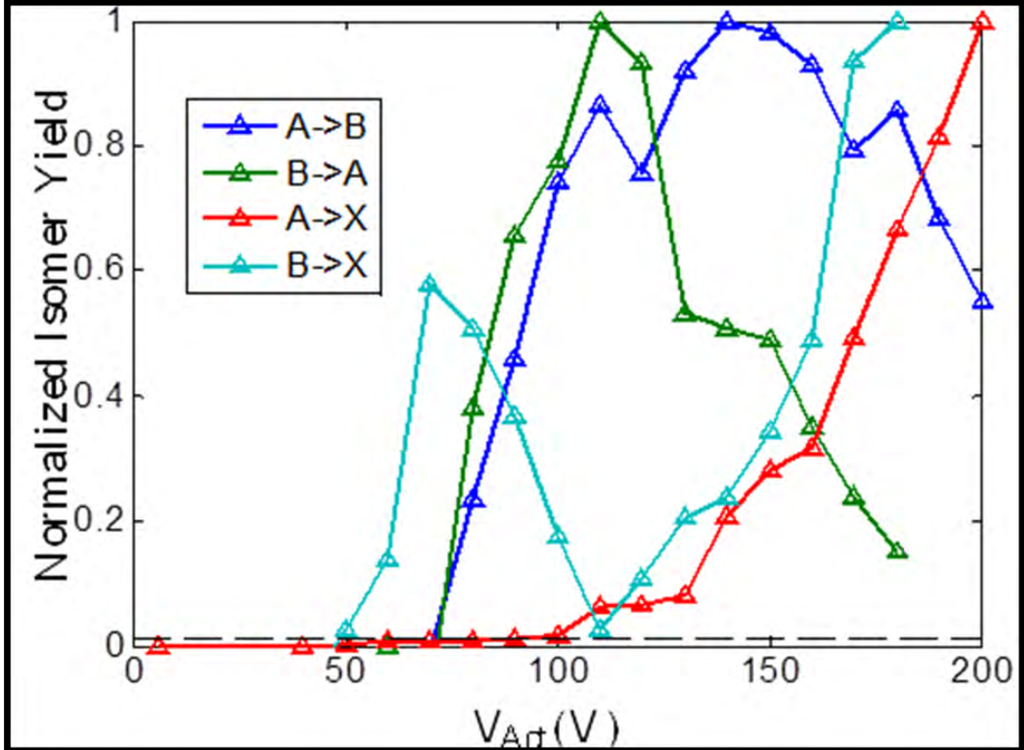


Experiment – Barrier energy in protein is ~1-1.2 eV, and inversely correlated with the absorption wavelength

Theory – ~1-2eV

Barlow R.B.; Birge R.R.; Kaplan E.; Tallent J.R. *Nature* **1993**, *366*, 64–66
Gozem S.; Schapiro I.; Ferre N.; Olivucci M. *Science* **2012**, *337*, 1225-1228

IMS-IMS-MS: $[RPSB+H]^+$



- State-to-state conformational transitions
- Fragmentation pathway through a more compact, intermediate species

Dilger J.M.; Musbat L.; Sheves M.; Bochenkova A.B.; Clemmer D.E.; Toker Y. *Ang. Chemie Int. Ed.* **2015**, *127*, 4830-4834